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PRODUCTION EXAMINATIONS ON PLANT ASSOCIATIONS OF GRASS-LANDS WITH SOLONETZ SOIL I. EFFECT OF CLIMATIC AND SOIL FACTORS ON DRY MATTER, CARBOHYDRATE AND NITROGEN CONTENTS OF ARTEMISIO-FESTUCETUM PSEUDOVINAE

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Included in the synoecological research program of the halophil vegetation of Pannonicum some investigations of production biology were made in 1968 on sodic grasslands; these investigations were connected with the International Biological Program. In Hungary the total area of halophil soil amounts to nearly 0,5 million hectares, the synoecological conditions of which are but partly known. In order to secure a proper fodder basis the intensified exploitation of these areas and the increase of the production of phytocoenoses become more and more urgent. Since the production of the plant stands is considerably influenced by climatic and edafic factors, our investigations were aimed at analyzing the effect exerted by these factors on solonetz grass associations. Within the total production the predominant species were particularly examined, since the environmental factors exert an unequal effect on the various species. The present paper describes the investigation results achieved on the *achielleetosum* stands of *Artemisio-Festucetum pseudovinae* growing in the highest zone of the solonetz steppes.

Materials and Methods

The investigated area was the large pasture of Csanádpalota, some 50 km. to the east of Szeged (Fig. 1). Beginning with April 1968 the surveying and the gathering of material were repeated every month. Simultaneously with our investigations the quantitative and qualitative changes caused by various fertilizer doses were also evaluated (Ábrahám-Bodrogközy, 1968. Pro to the laboratory analysis of the gathered material it was kept in sachets in a refrigerator so as to enable the water contents to be exactly determined. The material used for the analyses of contents was fixed within 3 hours after the gathering, at 105 C°, and desiccated up to a weight stability at 70 C°.

The total carbohydrate contents were determined with 2 per cent hydrochloric acid after 2 hours hydrolysis in hot water bath by means of the phenol-

sulfuric acid method according to Dubois et al. (1956). Photometry was made at 400 nm on a MOM 360-type photometer. The standard curve was plotted with pa saccharose.

The total nitrogen contents were determined with a minor modification (Horváth, 1965) of the method of Kelly et al. (1946) after decomposition in cc. H_2SO_4. Photometry was made at 400 nm on a MOM 360-type photometer. The standard curve was plotted with pa ammonium chloride.

The overground biomass production was measured at the full development stage of the plants (middle of June) with help of the three-dimensional overground production calculation we have successfully employed for years (Bodrogközy-Harmati, 1966). The biomass evaluation per season or per month, as suggested by Boer (1962) and others, belongs to our future tasks.

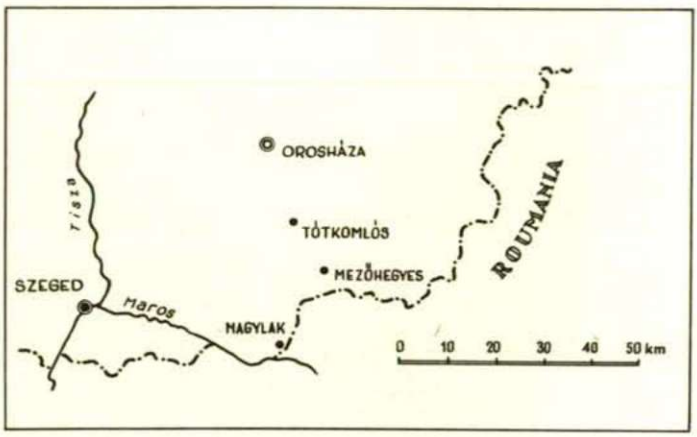

Fig. 1. Area of investigation and site of the meteorological stations.

Data of the nearest meteorological stations (Mezőhegyes, Tótkomlós) were used for the evaluation of the climatic factor (rainfall, daily average temperature as well as maximum and minimum values, number of sunny hours, etc.), while the value of the radiation energy ($cal.cm^{-2}.sec^{-1}$, measured with a Robitsch-type overall actinometer) was made available by the Climatological Institute of our University. Since there was hardly any difference between Szeged and Tótkomlós as far as the duration of sunshine was concerned, the energy values of radiation measured in Szeged could be fairly utilized in our investigations.

In order to analyse the effect of the soil factors the plant sampling was always combined with the exploration of soil profiles as far as 70—80 cm. depth. The humidity and granular size (graded to 6 fractions) of the soil as well as the concentration of Na salts were determined at every 10 cm. level according to the methods described by Ballenegger et al. (1962). Deeply situated subsoil water was left out of consideration because its effect is improbable (Ellenberg, 1952; Weise, 1954; Dancau, 1963).

Results

Due to the more favourable conditions of soil oecology (Ábrahám, 1967, — carbonate solonetz becoming steppe-type in deeper layers, the B_1 level is situated under 20 cm., low Na salt level of the leached A level) the glycophil and pseudohalophil species come into prominence with this type of Artemisio-Festucetum.

The stands usually include the following species:

Festucion pseudovinae species:

Festuca pseudovina v. *salina, Scorzonera cana, Limonium gmelini, Ranunculus pedatus, Trifolium retusum, Trifolium angulatum, Artemisia monogyna.*

Agrostion species:

Taraxacum officinale, Alopecurus pratensis.

Neutral species:

Cynodon dactylon, Gypsophila muralis, Achillea collina.

Festucion sulcatae (rupicolae) species:

Scilla autumnalis.

Even in case of the *Achillea collina* subassociation there is a rather poor combination of species to be found. — The frequent occurrence of a preglacial loess steppe element, *Scilla autumnalis,* may be regarded as a floristical curiosity. In connection with the changing of local factors it will be reasonable to submit the specific combination to an analysis per aspects.

Medium stage of the spring aspect (date of examination: April 26th 1968).

Climatic factor: The weather of the period prior to the gathering was substantially different from the average of many years. In the first two months of the year the number of sunny hours and the quantity of precipitation remained far below the usual values. Beginning with February the temperature was rising quickly; a daily average temperature of more than 10 C° was quite common. In March the number of sunny hours increased suddenly, with little rainfall.

Soil factor: In the profile of the carbonate-type grassland solonetz soil there was a thick A level, followed by a well developed columned level. It was only under 30 cm. where the total salt content surpassed 0,2 per cent, while it was somewhere about 0,1 per cent in the A level. The A level can thus not be considered as sodic soil.

Flora: The grass association was composed mostly of xero- and glycophil species. Relying upon character species it was found to be a transition between the associations *Achilleo-Festucetum* and *Artemisio-Festucetum pseudovinae.* The leached A level of a considerable thickness permits even several glycophil species to get established if they are able to adapt themselves to xerotherm local conditions.

The appearance of the *Agrostion* species by blades — such as *Alopecurus pratensis, Taraxacum officinale* — permits to conclude on a development from slightly sodic moorland. (Bodrogközy, 1960).

The effect of xerotherm local conditions is reflected also in the relative dry matter contents of the species. However, there are considerable differences between the species. So for instance the deeply rooted species, such as *Scorzonera cana* and *Taraxacum officinale* are poorer in dry matter, while *Festuca pseudovina* with no deep roots has a higher content of dry matter (Fig. 2).

Scorzonera cana has the highest N content, three times as high as that of *Taraxacum*. An inverse situation may be found in regard to carbohydrate contents (Fig 2). *Festuca pseudovina* has an outstanding carbohydrate content, which might probably be explained with the fastly developing raw fibre content.

After-stage of spring aspect (date of examination: May 9th 1968):

Climatic factor: Due to the effect of rainfall, to the rising number of sunny hours and to the temperature maxima of about 30 C° before the gathering, it had hardly any effect.

Soil factor: Beginning with the spring aspect, the little atmospherical precipitation caused a definite xerophil character. As far as the depth of 20 cm. the soil humidity was less than 10 per cent. Due to the physical structure of the soil even this may be regarded for the most part as slack water.

Flora: The combination of species has slightly changed. The rainfall of 50 mm. prior to gathering has mainly increased the water content of *Festuca pseudovina* (cca. by 15 per cent), and to a smaller extent that of *Scorzonera* and *Taraxacum*.

The total N content of *Festuca* remained unchanged, that of *Achillea* has increased and that of *Scorzonera* has decreased. Carbohydrate contents have generally increased, particularly in case of *Festuca* (cca. by 15 per cent).

Early stage of summer aspect (date of examination: June 19th 1968):

Climatic factor: Beginning with the middle of June the number of sunny hours has considerably increased and was for several days even higher than 11. (It represented actually 95 per cent of all possible sunny hours.) Temperature was rising simultaneously. The third heat-wave of the 1968 vegetation period has developed immediately before the examination. The temperature maxima were about 30 C° and the minima were not lower than 20 C°. Increased transpiration was not compensated by rainfall since there was no important precipitation during the three weeks before the examination. (There was 40 mm. of rain between the two gatherings.)

Soil factor: The change in total salt contents was insignificant, but soil humidity decreased to 5 per cent in the upper layers. Mosaic-like cracks appeared on the soil surface.

Flora: Lively green even at the end of May in other years the steppe gradually dried out already from the beginning of May 1968 on. The vegetation had a yellowish greenish brown colour. *Taraxacum*, for instance, dried completely out and only the deeply rooted *Limonium gmelini* started growing. Due to the drought the plant association became poor in species.

The water content of the biomass decreased considerably; that of *Festuca pseudovina* was only 20 per cent and even that of the intensely

growing and lively green *Cynodon dactylon* was not more than 50 per cent.

The species *Scorzonera* and *Achillea* dried out to a lower extent (the comparatively higher humidity content of *Scorzonera* may be explained, among others, by the fact that most of the plants remained in the vegetative state on account of the drought).

While the relative dry matter content increased, the total N content diminished in every species. The slightest decrease was found in *Achillea*. It was rather remarkable that *Festuca* with many of its leaves becoming yellow had almost the same total N content as the lively green *Cynodon*.

As compared with the previous examination, the total carbohydrate content hardly presented any change.

After-stage of summer aspect (date of examiation August 1st 1968):

Climatic factor: Warm sunny weather since the last examination.
Soil factor: The total salt contents remained essentially unchanged. Due to nearly 80 mm. of rain in July the humidity of the superficial layers increased from 5 to nearly 20 per cent.
Flora: Until the rains came in the middle of July most of the vegetation became latent. (Even almost 40 per cent of *Limonium gmelini* was damaged.) Due to the temperature decreasing simultaneously with the rain (maxima of about 20 C°) the vegetation was restored to life. So the examination of August 1st presented almost the same species as that of June. Due to the regermination and the higher humidity of the environment, however, there was an important decrease in the dry matter content of the biomass to be observed.

The total N content of every species increased conspicuously and presented the highest values observed during the whole vegetation period. On the other hand, there was a decrease of the total carbohydrate content in every species except for *Cynodon dactylon* (especially in *Limonium* and *Festuca).*

Early stage of autumn aspect date of examination: September 5th 1968):

Climatic factor: The weather of the 35 days passed since the last examination was characterized by favourable rain, light and temperature conditions.
Soil factor: The total salt contents continued to be unchanged. In spite of the sufficient amount of rainfall the soil humidity decreased in this period by some 50 per cent (this was probably the consequence of the considerable water uptake caused by the intensive germination and growth of the plants).
Flora: With the reappearance of the *Taraxacum* and of some *Papilionaceae* species the specific combination was changed. Due to the favourable local conditions the water contents of the plants generally increased. This increase was particularly important in *Cynodon* and *Festuca.*

Among all of the examined species *Scorzonera* was the only one with unchanged water content.

Particularly in case of *Limonium* and *Achillea* there was a considerable decrease of the total N contents. However, the decrease of N contents was less important in the grasses yielding the bulk of the phytomass, especially in *Festuca pseudovina*. Except for *Limonium*, the decrease of total carbohydrate contents was equally important.

After-stage of autumn aspect (date of examination: October 11th 1968):

Climatic factor: The first two-thirds of the 35 days passed since the September gathering were extremely rainy (nearly 80 mm.). Notwithstanding, the number of sunny hours was rather high.
Soil factor: As compared with the last examination the soil humidity has increased, but in spite of the heavy rainfall it was hardly higher than 15 per cent even in the upper soil layers.
Flora: The specific combination hardly changed; the percentage of the *Papilionaceae* increased, while *Taraxacum* disappeared. The dry matter content increased considerably in most of the species, except for Festuca. Especially the relative dry matter content of *Cynodon* grew larger (cca. by 40 per cent).

As compared with the results of the September examination, the γ/mg values of the total N contents decidedly decraeased in every species, while those of total carbohydrate contents presented a considerable increase.

Summary

Included in the International Biological Program, synoecological and production investigations have been carried out in the vegetation period 1968 on sodic grasslands to the south-east of the river Tisza. These investigations were concentrated on the associative conditions of the Limonium facies of *Artemisio-Festucetum* pseudovinae as well as on the overground relative dry matter, total N and carbohydrate contents of the species, in the function of climatic and edafic factors. The following conclusions were reached:

1. At the beginning of, and prior to, the vegetation period the weather resulted in dry and warm local conditions; their effect was intensitied by steppe-type solonetz grassland soil with saline deep layers.
2. The plant association was poor in species, most of the ephemeral *Trifolium* species characteristic of sodic soil were missing *(T. retusum, T. angulatum, T. striatum)*, and therefore the investigations were mainly restricted to *Festuca pseudovina, Cynodon dactylon, Scorzonera cana, Achillea collina* and *Limonium gmelini.*

3. The total salt contents of the upper 60 cm. soil layer hardly changed during the vegetation period and was unaffected by rainfall.
4. The water contents of the upper soil layers was more intensely influenced by the condition of the flora than by rainfall.

5. The relative dry matter contents of the examined species changed in a considerable degree and unlikely to each other. In *Festuca* this change was as high as 100 per cent. The relative dry matter content is in close correlation to the rainfall conditions. The slightest change can be observed in the relative dry matter contents of deeply rooted species *(Scorzonera, Limonium).*
6. Total N contents reach a peak value during the regermination at the end of summer and are particularly high in *Papilionaceae, Limonium, Scorzonera* and *Achillea.*
7. The change in the total carbohydrate contents was less important. Outstanding carbohydrate contents were observed at every examination in *Festuca pseudovina* and *Cynodon dactylon.*
8. In general the total N and carbohydrate contents changed in opposite sense.

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Fig. 2. Change of the nitrogen and carbohydrate γ/mg of the total dry-material content over soil surface of the species *Artenmisio-Festucetum pseudovinae achilleetosum* during the vegetation period of 1968, in the proportion of the changes of climatic and soil factors.

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MANIFESTATION OF CHEMICAL AND BIOLOGICAL MOTION FORMS IN THE EXCEPTIONAL REARRANGEMENT OF MACROMOLECULES

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Introduction

The exploration of structure of the first expressively biological macromolecules: desoxyribonucleic acid, ribonucleic acid, myoglobin, haemoglobin, has opened a new period in the science of biology. The effect of results of the molecular biology is of so wide spectrum that its importance and connections cannot be seen completely, as yet. The results so far may doubtless induce a justified satisfaction giving rise, in the same time, to real doubts, as well. It becomes necessary to generalize the results and to select the research directions that will solve the other essential problems.

As we strive to recognize the cell, we cannot help disassociating the living cell into more and more elementary components, investigating it with isolating methods. In the course of work, getting giddy with some important results, we can be under the impression that the regularities, properties of the elementary particles are summed up simply in the complex systems, i.e., the supra- and macromolecular motion forms can be reduced entirely to the molecular motion forms. The exaggerated enthusiasm and preponderance of those thinking mechanistically is involving the danger of forgetting possibly the truth that "it is proved by any state of substance, from the simplest one till the most complicated state, that the structure is a basic condition of the formation of the whole" (Svidersky, 1963).

In our former monograph (Maróti, 1967) we dealt with specificity of the structure of living beings, with relation of structure and function, the development of its idea. We have emphasized that the structure of living beings is not only the quantity and the simple qualitative relation of their constituents but it is also a definitely directed system of a complex interaction of their elements, being a foundation of their development, their relative stability and qualitative determination. In the introductory part, already, it is to be emphasized that, in contra-

distinction to papers of more authors: Mamzin (1960); Svidersky (1960); Meerson (1964), etc. and in agreement with that of Rózsi Varró (1966), we are endeavouring to disclose the specificity of the structure of living beings.

In our present monograph, we are looking for the connection in the creation of macromolecules that has induced the production of biological motion forms out of the chemical ones. We think to recognize that, in the structures of different levels but connected genetically and being exceptionally rearranged.

I. The structure is an exceptional and necessary rearrangement of the components of living beings

A pioneering work concerning the extensive and comprehensive disclosure of the structure as an internal content of the quality has been performed by Svidersky. First of all the results of organic chemistry, atomic physics and biological sciences have contributed to the constituents of structure being recognized. In the improving analysis of the qualitative determination the works of Holderith-Magyaródi (1963) are eminent, in the field of chemistry those of Erdey-Gúz (1967), and in philosophical connections those of Rózsi Varró (1966).

From the development of motion forms we may draw the conclusion that in the structure of living beings there must be something specific, some law of general validity, separating them from, and simultaneously connecting them to, the inanimate world. We think to recognize this peculiarity in the relation, of a direction exceptionally arranged in time and space, of the constituents forming the structure of living beings.

1. The origin of being exceptionally arranged

It is shown by the dialectics of the parts and the whole that it is characteristic of any states of substance to be arranged. The structure of living beings shows an exceptional arrangement differring from that of the anorganic world but the extraordinary foundation of arrangement is in the inanimate world. The question arises: How is manifested the arrangement in space and what is its cause? Whether the arrangement has quickly originated from the material performing an inferior motion and being generally arranged — or the foundation of the exceptional arrangement can be found already on sub-atomary, atomary and chemical levels, as well.

For a long time it has been a generally known fact that the organizing role of the constituents of structure of the living beings is not corresponding in value. Nevertheless, we have not noticed for long that in the development of the structures of different levels the levels, the new qualities are not originating frontally but owing to primary changes given by the internal feature of a few elements that determine the phenomenon. It is a natural consequence of these considerations that the foundation of a superior arrangement that is characteristic of

the structure of living beings can already be observed on sub-atomary and chemical levels, as well. Thus the interest turns repeatedly and first of all on the carbon atom. Then we have to look for a property of the carbon atom that is common in the course of the arrangements on different levels but it is manifested in different relations in the physical, chemical and biological motion forms.

From the 103 elements recognized so far, carbon is alone able to a replication consisting of more than two identical elements. As we use the notion of replication in a general form, it is desirable to define it exactly.

2. Notion of replication

Replication is a repetition of comparatively homogeneous elements (particles), being energetically interdependent and of nearly identical pattern, in which the first elements of the structure have a directing, determining ability. Replication has two main forms: structural replication — where pattern and copy remain in a strict connection in space — and free replication where the connection between pattern and copy is but temporary.

As a consequence of the replication, from the identical elements there are produced qualitatively new "aggregations" the basic unit of which is two or four. In respect of arrangement, pattern and copy are nearly equivalent but as to the ontology of the phenomenon, the pattern is primary.

In case of structural replication it seems so as if pattern and copy were entirely coincident with each other, i.e., as if the identical elements could form an accidental or discretional aggregation. Actually, in the course of replication the identical elements suppose each other reciprocally, they may be potencially patterns and copies of one another.

As a result of the replication, besides the quantitative conditions, there prevail more and more the complicated and increasing reciprocal effects in the new structures produced, forming the internal content of the new qualities and, at the same time, a foundation of the exceptional arrangement.

3. Characteristics of the exceptional arrangement

After all these we should investigate by what properties the exceptional arrangement in space and time of the different forms of material is shown.

a) A connection of the parts forming the phenomenon in which these parts are capable maximally of preserving and changing their own features.

b) The few elements of the phenomenon that have preserved in an increased degree their homogeneity can change, primarily and directing, as a result of the other elements of the phenomenon.

c) The exceptional arrangement is a manifestation of the general arrangement, a result of the selecting influence of the inorganic world.

d) An aggregation of the configurative energy is a condition of this rare rearrangement.

Ernst, reviewing (1963) the enthropy theorem formulated by Boltzmann-Planck ($S = k \ln W$), is pointing out that the absolute enthropy value consists of the thermic and configurative sums of enthropy: $S=S_k+S_t$.

The mathematical meaning of the law expresses the superiority of the statistical distribution in contradiction to a rare arranged distribution, as well the reciprocal relation of structural arrangement and enthropy. It is obvious, therefore, that from the elements recognized so far, only carbon is able, decisively and determinatively, to accumulate the configurative energy on molecular level (decreasing entropy).

The exceptional arrangement manifests itself in different forms on different levels of development of the organic matter; anyway, every level is conform to the others in being a result of replication and in the reductibility of the potential basis of arrangement to the nuclear structure of the carbon atom.

II. Role of the carbon atom in the development of the exceptional arrangement

We are surrounded everywhere by living beings. Examining our surroundings and ourselves, we may become victims of optical illusions, namely it can seem so as if the biological motion form were a very general one. This is true, however, according to our present knowledge, only on the Earth and on similar planets. Erdey-Grúz (1967) is ascertaining that "the overwhelming majority of the substance of the Universe (in our Galaxy about 97—98 p.c.) don't participate in the chemical motion form". This statement is even more valid to the biological motion forms.

Structure and motion form of substance depend on temperature, pressure, on reciprocal effects of electricity and gravitation:

a) In a temperature of a few hundred thousand C°, under a pressure of some million atmospheres there are neither molecules nor atoms, in the so-called plasma state there are to be found mostly bare nuclei and freely moving electrons between them.

b) In some ten thousand C°, under a pressure of a hundred thousand atmospheres more and more electrons join gradually the nuclei: new quantized qualities, the atoms are produced.

c) In about 12—10 thousand C°, under a pressure of a few ten thousand atmospheres attraction and repulsion between the atoms begin to show themselves. The chemical motion form, however, becomes essential only under 5000 C°.

d) The majority of the organic compounds decompose, are reduced to carbon under 300 C°, and even the comparatively stabler compounds of small molecular weight about 700 C°.

e) In case of living beings, death comes generally at about 50 C° (in case of blue algae of thermal springs, of some bacteria, at 70—75 C°),

and about the freezing-point, the majority of the vital processes cease to be.

Taking all these into consideration, we can ascertain that life is a very rare phenomenon that is a result of the increasing internal and external reciprocal effects of the elements composing the structure (of the increasing sensitivity to heat and pressure). And we again get to the basic point of departure, whether every element (atom, molecule, macromolecule, supramolecule, organellum, etc.) is taking part, as equal, in the increasing mutual effect or only one or a few of them is playing decisive role.

Whether or not the rather vulgar fantastic idea according to which in the colloidal material of some space living beings there is silicon or germanium instead of carbon, and in their energy systems arsenic or sulphur instead of phosphorus, has a real basis?

It follows from the notion of the exceptional arrangement that this real basis is missing. Life could develop only by the primary and directing organization of the carbon atom. If there are living beings on a planet of Universe, the external conditions and their development must be similar to, the internal organization of the living matter must be identical with, those on the Earth.

Before beginning to analyse the development levels of the exceptional arrangement in details, an important question is to be cleared that may have already arisen many times in the reader.

Isn't it a metaphysical speculation, to establish any connection between the organization and function of structure of the living beings and the structure of carbon nucleus and electron shell? In our opinion, it isn't.

If we emphasize only the external interactions (relation to one another and to other molecules) of a given structure (e.g., DNS, ATP, etc.), so we separate its main motion form (function) from its accidental motion forms, from the regularities which are valid in the relation of its constituents, remaining subordinated even in the structure of higher level. So the genetical connection existing in the structures would be neglected, the structures of different levels would be separated mechanically, seeming to be without any correlation what is fully opposite to the evolution of the living world. This is made clear by H e g e l (1957) in connection with the formation of a new thing: "The basis of the origin being gradual is the idea that the thing which is about to come into being is already existing actually sensorially or generally only it isn't perceptible, as yet, owing to its small size; and the situation is the same at a gradual fade-out: the nonentity or something else which will substitute it is already present in the same way, without being anyway perceptible for the time being".

It is similarly a metaphysical way of thinking if in a given structure we are emphasizing only the internal reciprocity of the elements getting, in that way, to the false opinion that the motion form of higher order can be reduced from that of lower order. That is to say, in the structures of different levels there isn't any new quality, they are simply added together mechanically from components (structures) of lower level — what is similarly at variance with experience.

1. Specificity of the carbon atom

It is ascertained by Erdey-Grúz (1967) that: "the chemical motion form is connected with the interaction of atoms themselves, its phenomena are the manifestations of internal contrasts showing in the specific (nongravitational) attraction and repulsion".

In the interrelation of carbon atoms an exceptional contrast is showing, as a result of which from the 103 elements only carbon is able to change its properties preserving them, anyway, maximally. What is the cause of that? The replicative structure of carbon atom:

a) Carbon is the first element of the fourth "b" column of Period Two. The mass number of the atom (A) is formed by the amount of even-numbered protons (P) and neutrons (N). The atomic number (Z) and the number of protons is identical $A=P+N=Z+N=6+6$.

b) The origin of carbon nucleus may have been based on the principle of replication. This seems to be verified by the collision of the first element (Berillium) of the second column of Period Two against an α particle:

$$^{9}_{4}\beta e(\alpha, n)\, ^{12}_{9}C$$

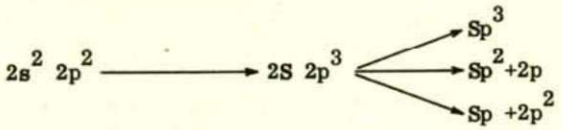
c) The proton-neutron configuration can form a nearly ideal symmetry. In Period Two, the cubical content of grammatom is the smallest, the nucleus having a great influence over the electron shells.

d) According to Bohr-Sommerfeld's theorem, the electron shell of carbon in fundamental position has the most homogeneous structure of replication that is nearly ideally symmetrical. The spherical K and elliptical L main quantum orbits, as well the main, subsidiary, magnetic and spin quantum values are in a relation of progressive replication:

Table 1

Z		K	L		Electron configuration
		1S	2S	2p	
1	H	1			$1S$
2	He	2			$1S^2$
3	Li	2	1		$1S^2\ 2S$
4	Be	2	2		$1S^2\ 2S^2$
5	B	2	2	1	$1S^2\ 2S^2\ 2p$
6	C	2	2	2	$1S^2\ 2S^2\ 2p^2$
7	N	2	2	3	$1S^2\ 2S^2\ 2p^3$
8	O	2	2	4	$1S^2\ 2S^2\ 2p^4$
9	P	2	2	5	$1S^2\ 2S^2\ 2p^5$
10	Ne	2	2	6	$1S^2\ 2S^2\ 2p^6$

e) The nearly ideal symmetry in ground state of the electron cloud of C atom becomes strongly asymmetrical as a result of the polerizing effect of the surrounding atoms; this asymmetry is the basis of a symmetry of higher degree. According to Panling)(1928), in the state of energized electrons of the carbon atom the $2S^2$ electron-pair dissolves: one of the electrons remains on the 2S spheric symmetrical orbit, the other on the 2p orbit takes up a dumbbell symmetrical orbit, forming a right angle to the two electrons there. This carbon atom joins with four, three or two atoms, the orbits of its valence electrons change, by being mixed ,into hybrid orbits oriented tetrahedron-like (Sp^3), plane-trigonally (sp^2) and linearly (sp):



f) The special attraction and repulsion between the carbon atoms change, as a result of the surrounding atoms, dynamically, through relative equilibria. The charge clouds of the hybrid orbits of contrary spinstate and of nearly identical energy level and those of $2p_z$ orbits penetrate into each other in different degrees. As a consequence of that, the value of the bond length characteristic of the molecular orbits of δ and π types is different:

Bond	Bond length A°	Occurrence
C-C	1,36	diacetylene
C-C	1,42	graphite
C-C	1,46	methylacetylene
C-C	1,47	butadiene (1,3)
C-C	1,54	paraffins, diamond
C-C	3,35	graphite
C=C	1,33	aethylene
C=C	1,37	butadiene (1,3)
C≡C	1,19	diacetylene
C≡C	1,20	methylacetylene
C≡C	1,21	acetylene

III. Compounds essential from the point of view of biostructure and function

General characteristics of the organic molecules composing the living beings — besides their properties enumerated above — are: an inclination to a structural heterogeneity, the macromolecules formed by the specification of the atom and elementary units, the structural energy, the increased sensitiveness to heat, the low speed of reaction. The carbon, preserving its homogeneity, is able to get into an electron-bond connection with elements of similar electron shell (H, O, N, S, P)

and, in that way, to change directingly and primarily concerning the whole organic molecules: The extremely various macromolecules, that are often species specific, are built out of a few elementary units, on the principle of replication.

1. Active, elementary organic "molecules"

In the course of the genesis of the natural organic molecules, the carbon atom, owing to its "inclination" to an exceptional arrangement, is capable of changing through minimal alterations-preserving its properties. The minimal alteration is assured by its electron-bond connection with oxygen, the next element of similar electron shell. The electron shell of oxygen is built up, as well, on the principle of replication ($1S^2$ $2S^2$ $2p^4$), it is, however, more mobile and its electron affinity is greater than that of carbon. Taking into consideration the replicative configuration of the electron shell, oxygen has the greatest capability of being polarized.

The primary elementary particles (ions, free radicals, elementary "molecules") that take part in forming the macro- and supramolecules of the structure of living beings are mostly derivatives of carboxylic acid. From the active carbon compounds the derivatives of acetic acid are particularly important. The electron configuration of acetic acid is determined, apart from being polarized inductively, by the configurative interaction of electrons and of the solitary electron-pair.

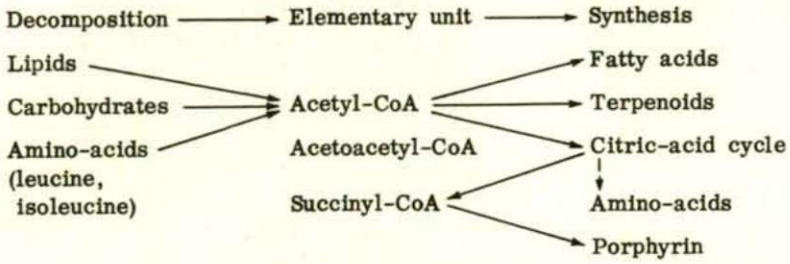
In forming and building the structure, also sulphur that is in a close connection with carbon, has a considerable role. The electron shell of sulphur atom, as well as that of oxygen, are built up in a replicative way ($1S^2$ $2S^2$ $2p^6$ $3S^2$ $3p^4$). Its polerizing and conjugative interaction is, however like that of oxygen.

For demonstrating that in synthesis of the biostructure the exceptional arrangement prevails, it is necessary to reveal the replicative elementary units. Decker and Lynen (1951) have discovered the acetyl-CoA enzyme. They have demonstrated that in the living organism the acetic acid is forming thiol-ester, not in its free shape but the acetyl group with the sulfhydryl group of the coenzyme-A:

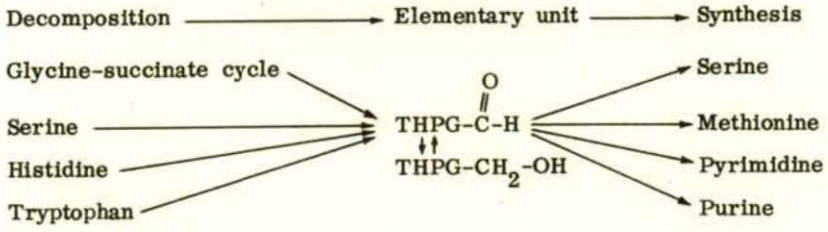
$$CH_3\text{-}\overset{\overset{O}{\|}}{C}\text{-S-CoA.}$$

Since then, it was often demonstrated that the active C_2 compound takes a central place in metabolism.

In the living organism there are a lot of amino-acids and nucleic-acids containing odd numbers (3,5) of carbon atoms. These compounds are mostly formed through a replication of the active C_1 radical (N^5 or N^{10}-formil tetrahydropteroilglutamic acid, THPG) and of the active C_2 or C_4 compounds.



Place of the active C_2 and C_4 compounds in the synthesis of molecules forming the biostructure of cells



Place of the compound C_1 in the cell metabolism

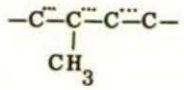
2. Fatty acids

In the formation of the boundary membranes of the cell the lipids have a great importance. Most lipids contain fatty acid in ester-bond. All the fatty acids occurring in the living organisms have even carbon-atomic numbers. In the various organisms there occur mostly fatty acids of 16 and 18 carbon-atomic numbers. The biosynthesis and decomposition of fatty acids are among the most important proofs of the manifestation of the exceptional arrangement. Taking into consideration that the fatty acids are mostly of dimer structure proved by the hydrogen-bond formed between two molecules, so the replicative unit of four carbon-atomic number has a manifold verification.

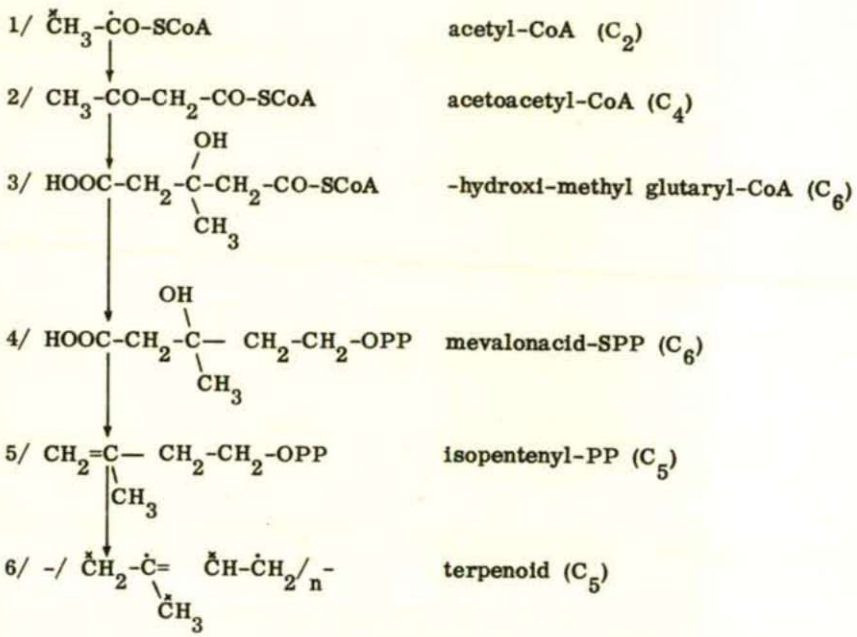
3. Compounds of isoprene skeleton

The terpenes (ether oils, phytol, K and A vitamins, carotinoids, steroids, caoutchouc, etc.) are compounds rather general in the flora and fauna. The terpenes are characterized by being constructed of the isoprene structural unit. A property of the isoprene skeleton is that the body of an "elementary molecule" is formed by four carbon atoms in an sp^2 plane-trigonal hybrid state that are connected with π delocalized conjunctive mutual effects induced by δ-bond and p-electron orbits

orientated at right angles to it. The reflection symmetry of the four carbon atoms is decomposed by the electron-repulsive methyl group connected with δ-bond to the double carbon atom.



The unit containing five carbon atoms seems to be in contradiction to the principle of replication, i.e., the C-atomic numbers 1, 2, 4, 6 are to be expected as a structural unit. In the course of studying the production of terpenes, however, it was ascertained by Grob (1951), Decker and Lynen (1960) that the active C_2 compound, acetyl-CoA, is one of the precursors of isoprene, as well. The major parts of the bi-synthesis of terpenoids are summed up, on the basis of the works of Lynen (1959), Tada (1961), Sandermann (1962), as follows:



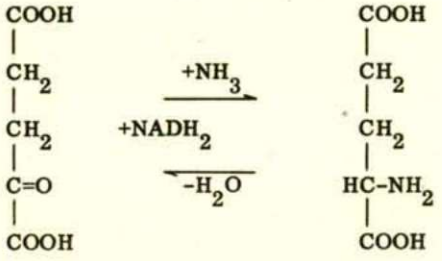
4. Amino-acids

In the genesis of macro-molecules, besides the relation of carbon with oxygen also its connection with nitrogen, the other element of a similar electron shell, is of decisive importance. Oxygen is the activating, motile "relation", nitrogen is the most consistent with carbon, the organizing, directing element. Besides the great importance of nitrogen, Doby's (1941, 1959) opinion is one-sided saying that N is the "vital

element" of cells, so-called governing element. Carbon, getting into an electron-bond connection with nitrogen, preservs and partly disintegrates its hegemony and is, therefore, able to direct syntheses in which the elementary particles change preserving, anyway, their own properties in an increased degree.

In forming the macro-molecules, the decisive process in the first degree is the synthesis of amino-acids. To take nitrogen into an organic compound can be connected with the inductive polarizing ability of oxygen. A decisive evidence for the amino-acids being synthetized by replication would be if we could establish which was the very first elementary compound, amino-acid, possibly amino-acids that the other amino-acids can be derived of. The great lot of experimental data collected in the latter decades don't give any satisfying reply to this question. This can partly be explained also by the fact that the bulk of experiments took place with heterotrophic organisms, first of all with animal objects.

It is a general opinion that in the synthesis of amino-acids glutamic acids play the primary role. This opinion is supported by the primary appearance of α-keto-glutamic acid in Szentgyörgyi-Krebs's cycle and by the reversible transformation of NAD with co-enzyme into glutamic acid.



The central significance of glutamic acid is still more emphasized by Cohen's and Cammarata's (1950) remarkable results according to which they could transaminate the synthesis of 20 sorts of amino-acids with α-keto-glutamic acid. In spite of several convincing results, in our opinion the primary amino-acid is not the glutamic acid but aspartic acid:

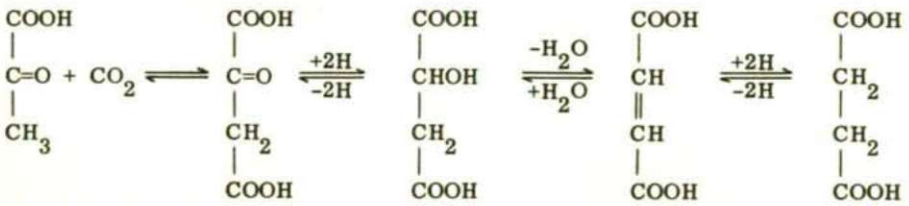
a) Glutamic-acid is of 5 carbon-atomic number, therefore, it cannot be an elementary unit of replication.

b) In the course of photo- and kemo-synthesis, it appears later than aspartic-acid.

c) Glutamic-acid is produced first of all during dissimilation.

In the course of kemo- and photo-synthesis, the dicarboxylic acids that contain four carbon atoms can appear primarily, too, thus they take a central place in the synthetizing and decomposing processes, among others, in the primary formation of aspartic acid. C_4 acids are produced even at the assimilation of CO_2 in darkness. These acids were earlier considered to be productions of a decomposition. On the other hand,

Werkman and Wood (1936) supposed in case of bacteria producing propionic acid, and Krampitz and Werkman (1951) verified about them, that the enzymes of the citrate cycle synthetize succinic acid, functioning in a reversible way.



pyroracemic acid | oxalacetic acid | hydroxysuccinic acid | fumarolic acid | succinic acid

This process has a great importance for the primary formation of the active C_4, succinyl-CoA and aspartic acid. In genesis of the amino-acids, the primary appearance of aspartic acid has been established during the investigation of photosynthesis, as well.

Table 2. Percentage and temporal appearance of $^{14}CO_2$ in primary compounds produced during photosynthesis

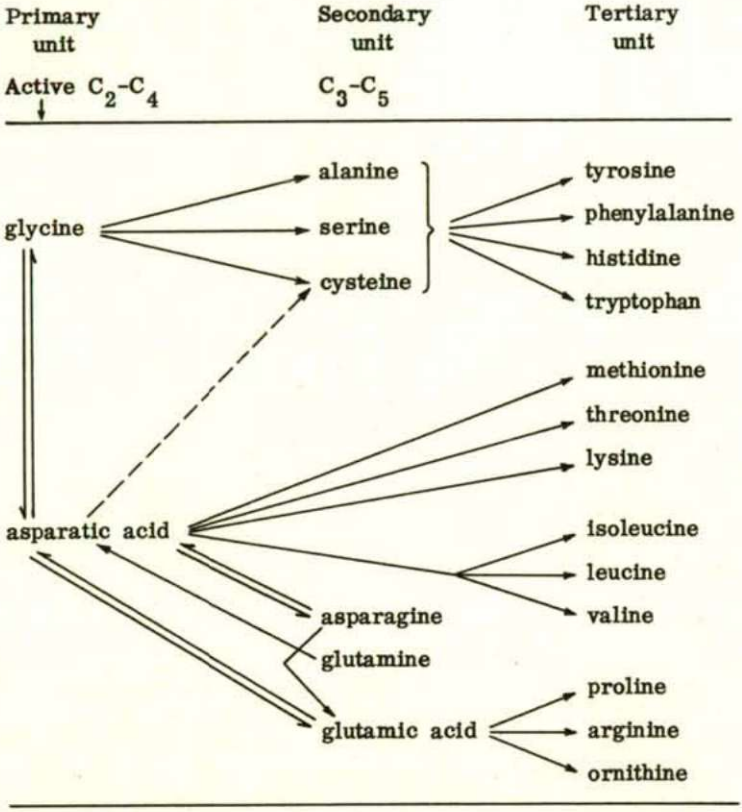
Investigated plant	Scenedesmus[1]				Chloroplast[2] + cell sap	
Duration of photosynthesis	5 sec.	30 sec.	90 sec.	5 min.	1 min.	30 min.
glycol acid	-	++	+++	+++	-	-
phosphoglycerate	+++++	+++++	+++++	+++++	61	78
hydroxysuccinic acid	+	++++	+++++	+++++	4,2	4,0
fumarolic acid	-	-	+	+	-	-
succinic acid	-	-	+	-	-	-
glycine	-	+	+	+	-	7,8
alanine	-	+	++	+	-	1,8
aspartic acid	+	+	++++	++	7,9	4,5
glutamic acid	-	-	-	+	-	-

[1] Signs according to Calvin and Benson (1948): - none, + hardly observable, ++ very weak, +++ weak, ++++ strong, +++++ very strong

[2] Results of Holm-Hausen et al. (1959) obtained from chloroplast suspension isolated of spinach leaves.

Aspartic acid is an active compound, turning fast into other amino-acids. Rautanen (1948) fed pease with aspartic acid and it became manifest that aspartic acid did not accumulate even in that case in a larger amount but it was changed into glutamic acid, glutamine and alanine. If this proved true generally in case of several plants, it would considerably support the theory of the genesis of amino-acids by repli-

cation. The origin of amino-acids is not known in several respects. Nonetheless, it is certainly not a rash conclusion if we think on the basis of literary data that the primary role in the origin of the amino-acids is played by the molecules C_2 and C_4:



The supposed replication relations of the amino-acids.

5. Biosynthesis of heme, chlorophyl and cyanocobalamine

The natural synthesis of the porphyrine skeleton of tetrapyrolic structure is a remarkable proof of the organic molecules of every living being originating in their main features in a unitary way. This applies particularly to the formation of the primary and secondary elementary "molecules". In genesis of the organic molecules, levels, points of jonction can be distinguished. The appearance of life is proved by complicated structures continuously and broken from the atomary level. However complicated these molecules are, anyway, numerically they are very limited as compared to all those molecules that could be produc-

ed with another rearrangement of the same atoms. The discrete parts (molecules) that don't prove the exceptional arrangement being continuous, are selected out, their importance in the formation of structure is but of minor value. On the basis of the investigations of Shemin (1962), Granick (1967) and others we know that both in case of plants and in that of animals, the porphyrine skeleton may be reduced to the replication synthesis of succinyl-CoA and glycine (active C_2 and C_4). The amphoteric porphyrine-ring is built up by 20 carbon atoms and 4 nitrogen atoms, forming a heterocyclic, conjunct aromatic system. The continuous conjunction extends over 16 carbon atoms and two nitrogen atoms. The specifity that is characteristic of the single molecules and taxons is assured by the change of a few elements, radicals, connected with the macrocycles.

IV. The increasing arrangement in space and time is a condition of the exceptional rearrangement of the elementary molecules

In our first monograph we have emphasized that the exceptional arrangement is multilevelled and that these levels are connected with one another. Erdey-Grúz (1967) establishes in connection with the rearrangement of matter that the three-degree classification of the rearrangement of matter (atom, molecule, macrobody) is already antiquated. The chemical substance is arranged in more degrees, through new material qualities. The formation and development of the living substance, as well, are realized through main stages and smaller points of junction.

The primary and secondary elementary units of organic compounds (free radicals, elementary molecules) originate spontaneously in the inanimate nature. The atomic groups produced in that way get into connection with one another, the direction of their reactions is reciprocal, in compliance with the laws of concentration and mass effect, pointing to a statistical equilibrium, while their entropy is growing. The parts, and only those ones that have preserved in an increased degree their exceptional arrangement in the course of their reactions, can temporarily get into a progressive dynamic equilibrium by using external energy through more points of junction, i.e., their structural arrangement is growing, their entropy decreasing. After having reached certain degree, however, they are no more capable of any further spontaneous arrangement and decomposed get into a static equilibrium. The elementary particles of carbon, oxygen, nitrogen content that in the structural heterogeneity have preserved their homogeneity in a higher degree get, primarily too, more frequently into a temporary dynamic equilibrium, increasing in that way their own directing, arranging, catalysing "inclination".

As a result of the increasing arrangement in space and time, there are produced units of primary, secondary, tertiary and n-th order in different amounts. The molecular number of the primary units is much higher than e.g. that of the units of the fourth order.

Each of the molecules comes, because of its internal structure, into conflict with the surroundings created by itself and by other molecules. The struggle of contrary tendencies is resulting in a peculiar distribution of molecules in space and time. The identical molecules accumulate more and more in space, getting localized. The temporary accumulation and absence of these elementary units induce an increasing rhythm.

The exceptional arrangement predominates over the particular relation of the elements, besides the characteristic quantitative changes. Without discussing any further interrelations of these elements, their determined symmetrical (right and left) and temporary connections, we only call the attention to the peculiarity of the quantitative connections.

On the different evolutionary levels of the exceptional arrangement. the elementary particles often form double and fourfold, rarely sixfold, fivefold and threefold elementary aggregations. Pressed for space, we are giving here only a few examples of the double and fourfold units:

1. General properties of the organic compounds playing an outstanding role in the structure of living organisms are the replication of the atomic and elementary atomic groups and a structural heterogeneity. The units of replication containing double and fourfold carbon atoms are the following:

a) In the cocoanut oil the percentage of fatty acids of different carbon-atomic numbers is: C_8=9 p.c.; C_{10}=8 p.c.; C_{12}=52 p.c.; C_{14}=19 p.c.; C_{16}=10 p.c.; C_{18}=12 p.c.

b) The "body" of the isoprene skeleton is formed by four carbon atoms. Phytol and vitamin A are consisting of 20, carotenoids of 40 carbon atoms.

c) In the ring of ribose, desoxyribose, xylose, arabinose, furanose, four carbon atoms form a unitary electron shell structure.

d) The dicarboxylic acid of four carbon atoms and the succinyl-CoA have a central role in forming the cell structure.

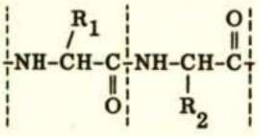
e) In the production of the amino acids, glycine and aspartic acid have supposedly a primary importance.

f) The porphyrine skeleton consists of quadruple units.

g) In the ring of cytosine, uracil and timine pyrimidine four canbon atoms, two nitrogen atoms form a conjunction unit.

h) The value of the 9.N-glycoside bondlength of adenosine and guanosine is supposedly smaller than 1,47 A°, thus the 1'-carbon atom of ribose β-configuration can belong, as to the electron distribution, to the furanose as well to the imidazole ring.

i) In the ridge of the polypeptide chain



units are repeated.

2. A general propriety of the macro- and supramolecular systems is the occurence of stereo-heterogeneity and the molecular replication.

The increasing of space-specificity is made possible by the formation of the primary filamentary, helical structures. The secondary and tertiary conformations are verified by the hydrophobic mutual effects, hydrogen-bonds, disulphide-bridges. During the molecule-replication or autocatalysis, pattern and copy are separated from each other. The first revolution of „independence" is a consequence of structural replication and of the increased arrangement in time. On this level, as well, the nx4 complexes are frequent:

a) DNS and RNS are formed by four bases;
b) Proteins are formed by 20 kinds of amino-acids;
c) Haemoglobin consists of four sub-units. The structure of the quadruple one is formed by two α- and two β-polypeptid chains;
d) The nucleic acid of the bacteriophage marked as Φx174 is covered with 12 protein molecules. On the nucleic acid of the poliomyelitis virus (pathogene of infantile paralysis) 60, on that of the Bushy stunt virus 120 protein molecules take place;
e) The pyruvate dehydrogenase multienzyme-complex is formed by 16 molecules decarboxylase, 64 molecules lipoic acid transacetylase and 8 molecules flavoprotein;
f) The cilium of the bacterium *Salmonella* is built up of four elementary filaments;
g) On the external side of the thylacoid membrane of chloroplast the multi-enzyme complexes take place in groups of 4 (quantasoma) or in those of 8, 12 and 16.

3. The basis of the exceptional arrangement of the living beings is the process-heterogeneity and the cellreplication. The structural organization gets a definite direction: it progresses from a relative homogeneity towards a heterogeneity, then it jumps to a homogeneity of higher degree. Owing to the characteristic "own space" and "own time" symmetry, rhythm, process of the cells, they get free gradually and more and more from the immediate influence of the inorganic world. The numerical observation of the cell division at the multicellular beings is difficult to follow after the fourth rhythm. The cell replication can be observed well in cell families living in loose colonies.

a) At some kindes of bacteria, e.g., in *Thiosarcina* and *Sarcina* colonies the nx4 location of cells can be seen well;
b) In the cell families of blue algae surrounded by mucilage-covers the change of cell numbers can be followed, as well: e.g., *Chroococcus turgidus* forms loose colonies consisting of 4 cells, *Merismopedia glauca* those of 32 cells, *M. punctata* those of 64 cells;
c) From the green algae, the species of the orders *Volvocales* and *Chlorococcales* afford the most beautiful and various examples of the colonies of cell-number 4;
d) Apart from enumerating still several examples, finally we mention that all the micro- and macrospores, as well ova and spermia, are generally formed in fours, rarely in nx4s.

In our monograph we have wanted to emphasize only a few aspects of the states of the exceptional rearrangement. With that, we should

like to emphasize. For being able to understand the idea of life and the laws of living matter, we ought to look in the inanimate and living world not only for differences but also for similarities.

The exceptional rearrangement manifested in the motion form of the supra- and macro-molecules is produced first of all by the free replication. The main role is played in the free replications by the van-der-Waals forces, in the structural replication by the chemical bonds. The free replication can be reduced genetically from the structural replication.

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COMPARISON OF THE LEAF EPIDERMIS OF SALIX ALBA L. IN DIFFERENT REGIONS OF THE LEAFY CROWN

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Introduction

In the course of investigating the leaf epidermis of amentiferous trees (Pataky, 1967), the question arose what an effect the conditions inside the foliage have upon some tissue elements of leaf epidermis.

According to Wagner (1957): "the more closed the foliage is the greater differences exist concerning the values and daily course of climatic elements, opposite to an open field". As demonstrated by Geiger and Amann (1961), the greatest fluctuations in temperature take place in the leafy crown; the greatest rise in temperature is found there. Kausch and Haas (1965) attribute the considerable difference, observed in the total amount of some chemical substances of leaves developed in the sunshine and in the shade inside the foliage of a free standing *Fagus silvatica* L., to microclimatic factors.

In the foliage, the different microclimatic conditions have the most direct influence on leaves supposedly through the epidermis. The question is whether or not there is a significant difference between the regions of leafy crown concerning some wellmeasurable constituents of leaf epidermis, i.e., whether the situation inside the foliage can be left out of consideration as diagnostized on the basis of epidermis.

With our investigations we wanted to get an answer to the following problems:

a) Whether or not the dissimilar ecological (or microclimatic) conditions of the leafy crown are reflected in some tissue elements — measurable exactly — of epidermis. (What is the number of stomata, length, width of guard cells, etc.).

b) As regards the single regions of foliage, which properties are changing?

c) In which properties is the degree of alterations significant and which are the comparatively stable properties of epidermis that can be used for diagnostic purposes, as well?

d) Is the reflection of the changing ecological effects of identical character and degree both in the upper and in the lower epidermis of leaf?

Materials and Methods

At selecting units and species, we have had regard first of all for the following points of view.

1. The ecological effects inside the leafy crown shouldn't be influenced by any conditions that doubtless occur in case of specimens from the same substance; therefore the material had been collected from trees standing alone.
2. The histological effect of the same ecological factors should be suitable for a simultaneous investigation in the lower, resp. upper epidermis of leaves.
3. We have compared fully developed, exactly determined leaves, resp. leaf parts with one another.

For investigating the conditions inside the leaf crown, we have selected a free standing specimen of *Salix alba* L. (Botanical Gardens, A. József University, Szeged).

Leaves (5—10 pieces) were collected from four different places of the leafy crown, for making preparations:

1. from the edge (outer part) of foliage
2. from the middle part „ „
3. from the inner part „ „
4. from the lower part „ „ out of

fully developed leaves between apex and base of a lateral branch. From the collected material there were made 2—4 epidermis preparations by maceration of every leaf, both from the lower and the upper surface of leaf (Fig. 1).

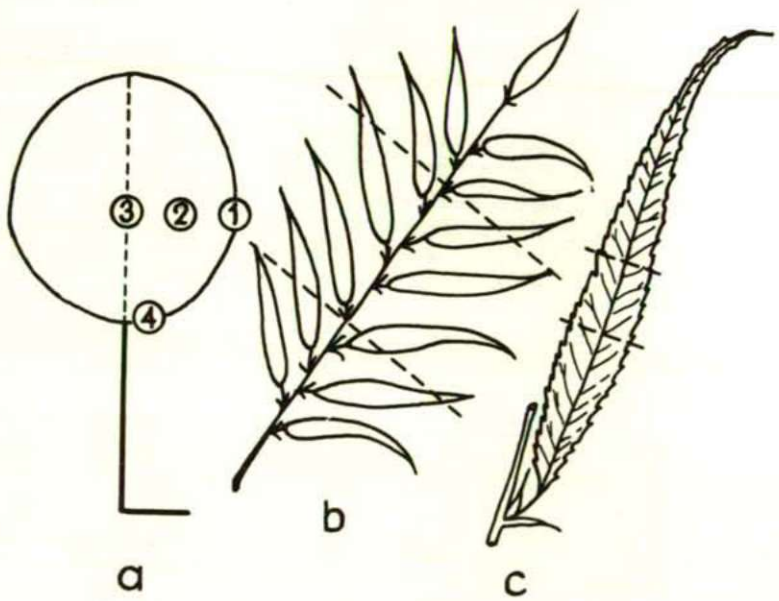

Fig. 1. *Salix alba* L. a) 1. edge of leafy crown, 2. middle of leafy crown, 3. inner part of leafy crown, 4. lower part of leafy crown;
b) lateral branch;
c) leaf pattern (the leaves) resp. leaf parts between the dotted lines are elaborated.

For staining the cleaned preparations, we have applied a triple staining with vesuvin, Ehrlich-f sour haematoxylin and Sudan III (Kisser, 1926).

We have measured the following tissue elements for comparison:

1. Number of stomata, piece/sq.mm (S).
2. Length of guard cells in μ (L). — (The greatest length of the two guard cells were measured in the direction of the longitudinal axis).
3. Width of guard cells in μ (W). — (The joint width of the two guard cells

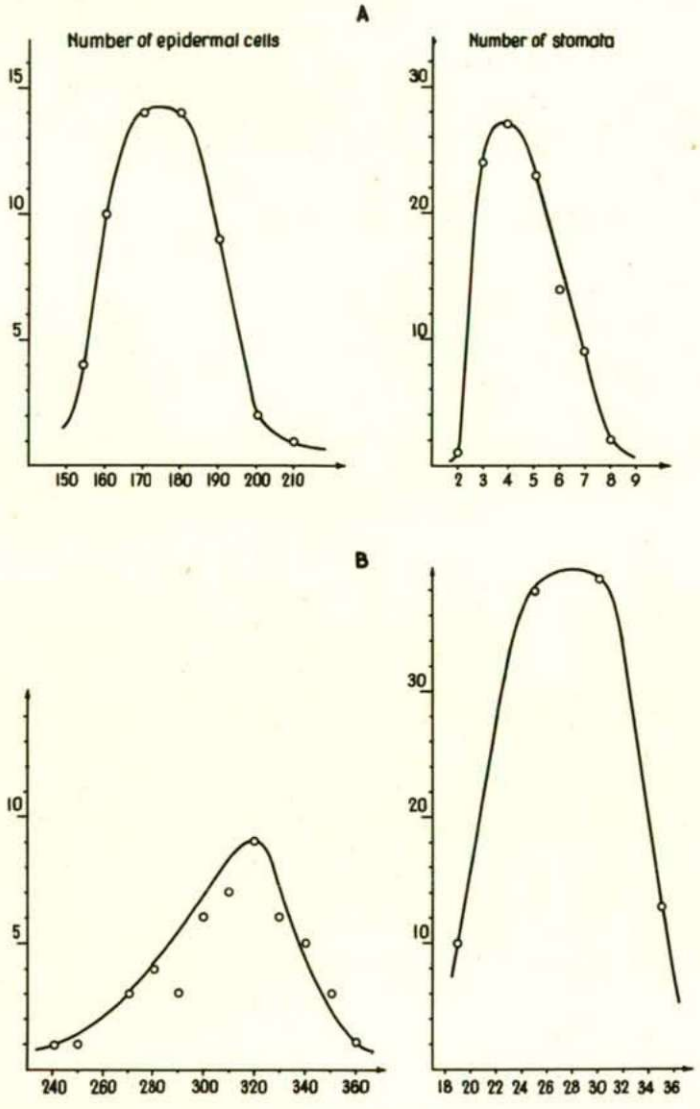

Fig. 2. Distribution curves of cell and stoma numbers of the epidermis, in:
A = upper surface epidermis
B = lower surface epidermis

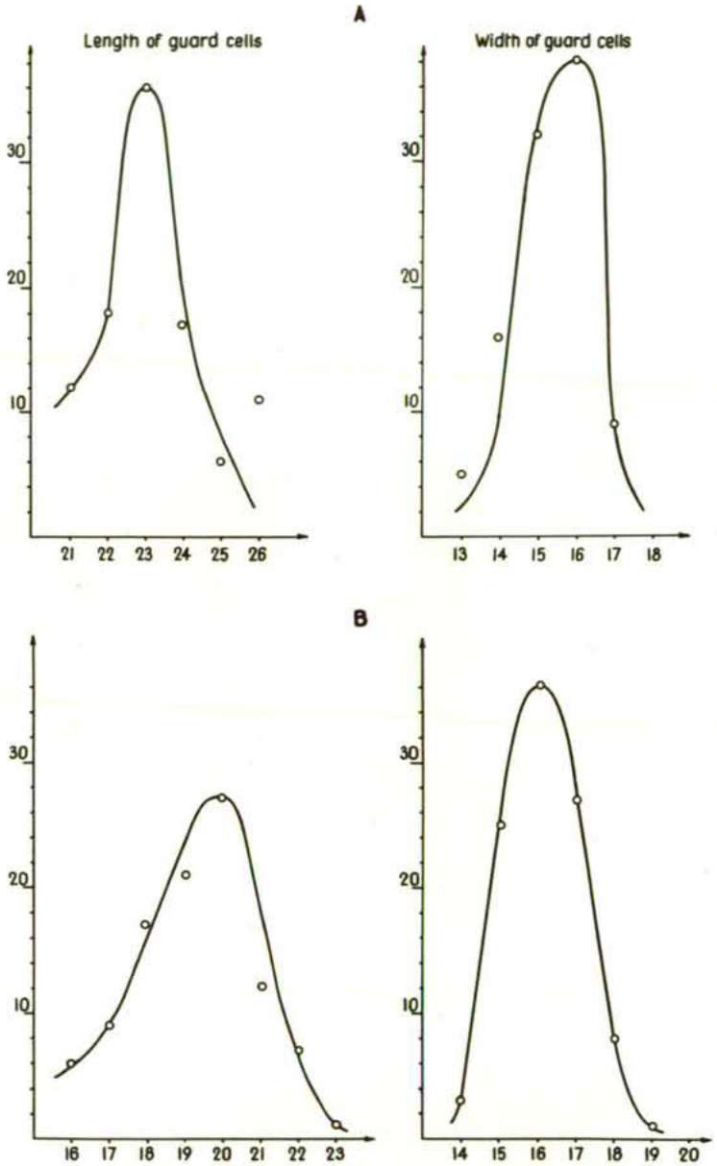

Fig. 3. Distribution curves of length and width data of guard cells, in:
A = upper surface epidermis
B = lower surface epidermis

were measured with the air slot between them).

4. Ratio length /Width of guard cells $\frac{L}{W}$ (A quotient of L and W of guard cells).

5. Number of epidermis cells, piece/sq.mm (E).

6. Stoma index ($I=\frac{S}{E+S} \cdot 100$).

The comparison of the properties enumerated above took place on the basis of the average values of data from 50 visual fields. (Measurements carried out with a Zeiss NF microscope, oc.x12,5, obj.x40).

The properties measured are showing a curve of normal distribution (Figs 2 and 3), the data are, therefore, estimated with variance analysis (Yule-Kendall, 1964). Calculations were performed by co-workers of the Laboratory of Cybernatics, Attila József University, with an electronic computer of type M:3.

Results

Structure and size of the epidermis tissue elements of *Salix alba* L. differ from one another on the upper and lower surface of the leaf. In the upper epidermis the cells are in every case larger than in the lower one (cf. Table 1).

The investigated properties of leaf epidermis are not homogeneous in the leafy crown, either. We have observed major differences between the single regions as to stoma number and epidermis cell number, and minor ones as to stoma index.

Size and quality of differences between the single regions of foliage are not the same in the upper and in the lower epidermis. The properties measured in the upper surface epidermis are differring generally significantly from one another, as regards nearly every region of the leafy crown. In the lower surface epidermis, the distribution of the same properties is more homogeneous, and in certain cases we can even notice some transition between the lateral and inner leaves of the foliage, e.g., as to stoma number (cf. Table 1). In the upper epidermis, there is a difference exceeding even a 0,1 p.c. SD-value between edge and interior of the leafy crown, resp. the lower and inner region of it. In the lower epidermis, only the difference between the edge and lower region of the leafy crown touches the SD level of 0,1 percent. Going from the edge of foliage towards the regions illuminated less strongly, the stoma number decreases gradually.

As to the cell number of epidermis, there are similarly essential differences between the single regions in the upper surface epidermis. There, the difference between the edge and middle of the leafy crown exceeds strongly even the SD-value of 0,1 percent.

Comparing the other regions of the leafy crown with one another, we find differences of 1 p.c. (edgeinterior of foliage) or 5 p.c. (interior-middle of foliage), except the middle and interior of foliage where the cell numbers are identical. In the lower surface epidermis, even between the most extreme values (lower part — interior of foliage), there is any difference only on the SD-level of 1 p.c. The differences between the

edge, middle and interior of foliage don't touch half the SD-value of 5 p.c., either. In these regions, therefore, the epidermis cell number is practically identical (cf. Table 1).

Table 1.

		Edge	Middle	Interior	Lower	SD		
		part of the leafy crown				0,1	1	5
						percent		
Stoma number	a	71	38	50	74	18,2	12,2	7,6
piece/sq. mm	b	231	199	171	152	67,6	44,8	30,4
Lenght of guard	a	29,7	29,7	26,5	25,9	2,2	1,4	1,0
cells in μ	b	25,9	24,4	23,3	25,0	2,4	1,5	1,1
Width of guard	a	21,1	19,7	17,7	20,7	2,0	1,3	0,9
cells in μ	b	17,5	16,8	16,6	18,1	1,4	0,9	0,6
Ratio L/W of	a	1,3	1,4	1,4	1,3	0,3	0,2	0,1
guard cells	b	1,4	1,4	1,3	1,3	0,3	0,2	0,1
Epidermis cell number	a	1823	1318	1545	1392	348,8	232,5	159,6
piece/sq. mm	b	2370	2340	2465	1783	683,2	455,2	312,4
Stoma index	a	3,7	2,8	3,2	5,1	1,4	0,9	0,6
	b	8,9	7,8	6,6	7,8	3,0	2,0	1,4

Alteration of tissue elements of the upper and lower surface epidermis of Salix alba L. inside the leafy crown. (a = upper surface epidermis; b = lower surface epidermis).

The stoma index is indicating the connection between stoma number and epidermis cell number, in a unit of field. In the upper surface epidermis, it is the smallest in the middle of foliage has the greatest value in the lower part of foliage the difference between the two extreme values being nearly double of the significance value of 0,1 p.c. In the lower surface epidermis, it is the smallest is the interior of folige; this value differs only from the stoma index of the edge of foliage on the level of 1 p.c.; it agrees on the other hand, with the stoma index of other parts of the foliage, in contradistinction to the upper surface epidermis.

The other properties investigated do not differ from one another in the most cases ,taking into consideration the situation inside the leafy crown. The results of our investigations are summed up in the following Table.

Evaluation of results

The stoma number in the single regions of the leafy crown shows significant alterations in the upper and lower surface epidermis. In the upper surface epidermis supposedly not the degree of illumination but

rather the water supply of leaves, the cc. of carbon dioxyde, the vapour content of air have effect on the stoma number. That is verified by the fact that at the edge and in the lower part of foliage, i.e. in places illuminated in a very different degree, there is no difference in stoma numbers; on the other hand, in the inner region of the leafy crown the stoma number decreases (cf. Table 1). In the lower surface epidermis, there is rather the decrease of the strength of illumination that has an influence on the stoma number, the most stomata being at the edge of foliage, that is to say, in the most illuminated parts; and the least of them are in the lower part of the leafy crown. [The effect of water supply and that of the single microclimatic conditions (light, CO_2 cc., vapour content) are interpreted, of course, in correlation with the morphogenesis of epidermis and of the leaves in different positions till the formation of the developed leaves.]

Summarized, therefore, on the basis of stoma number it is not possible to draw a conclusion of diagnostic value if the state of pattern inside the leafy crown is not known.

The length of guard cells changes hardly inside the foliage, only it is shorter in the interior of leafy crown. For diagnostizing, it is useful if the length of guard cells of the taxonomic categories under discussion is differring considerably from one another.

The width of guard cells between the regions of the leafy crown is showing a continuous transition: decreasing from the edge towards the interior of it (cf. Table 1). (The degree of the stomata being opened was left out of consideration. It is hardly suitable, according to our investigations, for characterizing the species.)

Ratio L/W of the guard cells — i.e., their shape, degree of their being globular — is not influenced by the conditions inside the leafy crown, either in the upper surface or in the lower surface epidermis. It can be used well for diagnostical aims if there are measurable differences between the single species.

The epidermis cell number falling to the unit of area is a function of the size of cells. According to Zalensky's law, the size of cells is decreasing parallel with an increase of the strength of illumination. As to the leafy crown, we have experienced during our investigations that the cells are bigger inside the foliage, i.e. in the regions less illuminated. The differences are of a considerable size between the parts of foliage illuminated differently, first of all in the epidermis of upper surface. The epidermis cell number can, therefore, not be used for diagnostic purposes.

The stoma index — the interior of foliage being left out of consideration — does not show any difference in the single regions, even on a 5 p.c. level. It can therefore be used for diagnostic aims as one of the complementary data. Our results concerning the formation of stoma number are, however, contrary to Zalensky's law, in view of the light conditions inside the leafy crown. Our statements about the stoma number are of course, reflected in changes of stoma index inside the foliage, as well.

Summary

We have investigated the leaf epidermis of a free standing tree (*Salix alba* L.) in function of the situation inside the leafy crown.

On the basis of our investigations it can be ascertained that:

1. Some tissue elements of epidermis change depending upon the conditions inside the foliage. A cause of alteration may have been the water supply of leaves being of different degrees and also the lasting influence of the different microclimatic conditions (light conditions, CO_2 cc., vapour content) prevailing in the morphogenesis (development) of leaves (Wagner, 1957; Geiger, 1927; Zalensky, 1964).

2. The degree (significance) of alterations is the greatest in stoma number and epidermis cell number.

3. The least extensive changes were observed in respect of the length-width ratio (L/W) of guard cells and of the length and width size of guard cells, as well in the stoma index. For diagnostic aims, therefore, the L/W ratio of guard cells, the stoma index, possibly as a complementary parameter the stoma number can be used.

4. Comparing the upper and lower surface epidermis of leaves, we have observed that on the upper surface the size of cells is generally bigger (cf. Table 1), the degree of differences found in the single tissue elements is, in the majority of cases, significant between every region. In case of using the epidermis for diagnostic aims, the lower epidermis of the leaf affords more reliable results.

My special thanks are due to Institute-leader Professor Dr. Imre Horváth for his kind instructions and to the collaborators of the laboratory for cybernetics for carrying out the computations.

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SPOREN-, POLLEN- UND MOORTYPEN AUS DEM MIOZÄNEN BRAUNKOHLENGEBIET VON NÓGRÁD I

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(Eingegangen am 25. November 1968)

Einführung

In dieser Abhandlung werden die im Laufe der palynologischen Untersuchung der miozänen Braunkohlen von Nógrád bestimmten Sporen- und Pollentypen angeführt. Diese dienen als Grundlage für die „natürlichen" Taxa, aus denen der Verfasser (1967a,b) auf das Helvetklima des Gebietes, den Ursprung und die regionale Verwandtschaft der Flora geschlossen hat. Wir versuchen in den Rahmen dieses Artikels die Braunkohlenmoore und die Ausbildung der Kohlenflöze von Nógrád aus den quantitativen Angaben dieser Flora, bzw. der Sporen- und Pollentypen zu rekonstruieren.

Material

Im Braunkohlengebiet von Nógrád hat Bartkó (1961—62) drei Hauptgebiete unterschieden:

a) Nördliches Revier von Salgótarján,

b) Mittelzone von Kisterenye—Mátranovák,

c) Südliches Revier, im nördlichen Teil des Mátragebirges aufgeschlossenes Kohlengebiet.

Der Kohlenkomplex im Gebiet besteht im allgemeinen aus drei Flözen.

Im nördlichen Revier hat sich nur das untere, Flöz III in einer Mächtigkeit für Abbau entwickelt. (Flöz II ist nur ca. 20 cm dick, und Flöz I ist nur mit einem pflanzenresthaltigen Sand vertreten.) Aus diesem Revier sind von uns nur 18 Proben der Unterbank von Flöz III des Schachts VI in Margitgrube (gekürzt: Margit III) palynologisch bearbeitet.

In der Mittelzone haben sich alle drei Flöze in einer Mächtigkeit zum Abbau entwickelt. Davon gerieten zur palynologischen Bearbeitung:

14 Proben von Flöz III des Schachts V in Újlak (gekürzt: Újlak III),

24 Proben von Flöz III des Schachts in Polyos (gekürzt: Polyos III),

14 Proben von Flöz II des Schachts in Polyos (gekürzt: Polyos II),
12 Proben von Flöz I des Schachts in Polyos (gekürzt: Polyos I),
18 Proben von Flöz II des Schachts II in Gáti (gekürzt: Gáti II),
18 Proben von Flöz I des Schachts in Pócsháza (gekürzt: Pócsháza I).

Im südlichen Revier haben sich nur Flöz I und Flöz II entwickelt, im Zeitalter der Bildung von Flöz III war das Gebiet noch viel zu trocken und erhebt, für Moorbildung ungeeignet. Davon stammen

11 Proben von Flöz II, Schacht II in Katalingrube (gekürzt: Katalinbánya II),
8 Proben von Flöz I, Schacht II in Katalingrube (gekürzt: Katalinbánya I),
21 Proben von Flöz I des Stollens in Ménkes (gekürzt: Ménkes I).

Die Probenahme geschah in Flözen Katalinbánya I und II nach Materialänderungen, in den anderen Lagerstätten in allen 10 cm. So zeigen die größten Probenummern eines Flözes gleichzeitig die approximative Mächtigkeit des Flözes in dm an (21 Proben=2,1 m).

Methode

Die Kohlenproben wurden mit HNO_3 und KOH, die viele Mineralien enthaltene Proben auch mit HF behandelt.

Aus den Präparaten haben wir pro Probe 100—200 Sporen, bzw. Pollenkörner bestimmt. Die originellen Mikrophotos sind in tausendfacher Vergrößerung mit einem Objektiv Zeiss HI 90 oder 100 verfertigt worden. Die Ergebnisse der qualitativen Analyse sind in dem folgenden Abschnitt enthalten.

Die quantitativen Angaben wurden in nach Profilen verfertigten, zusammengezogenen Diagrammen dargestellt (Abbildung). Detaillierte, die einzelnen Formen enthaltende Diagramme konnten leider wegen ihrer großen Ausdehnung in den engbegrenzten Rahmen dieser Zeitschrift nicht veröffentlicht werden.

Die Mikroflora von Nógrád

(Sporen- und Pollentypen)

Die im Braunkohlengebiet vorkommenden Sporen- und Pollentypen werden in der Reihe der Taxa des Soó'schen (1963) entwicklungsgeschichtlichen Systems angeführt. Wir geben eine kurze Beschreibung oder Diagnose nur bei den bisher noch nicht veröffentlichten neuen Typen. Zur Kenntnis der schon mitgeteilten Typen gibt das Literaturverzeichnis einen Anlehnungspunkt.

Die in den einzelnen Körnern vorkommenden Reste wurden, wenn sie unbekannt sind, nur bis zur Gattung determiniert.

Das Sporen-Pollenmaterial der Proben ist im allgemeinen synchron. Nur sehr wenig Pollentypen (*Trudopollis* sp., *Interpollis* sp.) und nur in einigen Exemplaren, stammen aus prämiozänen Sedimenten.

Es wird keine Tafelerklärung gegeben. Die laufenden Nummern der Sporen- und Pollentypen stimmen mit den Nummern ihrer Photos überein. Die Mikrophotos sind einheitlicher Vergrößerung (hier ca. x750); Maßstab auf Tafel I.

Bryophyta

Anthocerotaceae

1. *Anthocerisporis magnireticulatus* (Sics. 1964) W. Kr. 1967 (Photo 1).

Sphagnaceae

Sphagnum

2. *Sphagnumsporites stereoides* (R. Pot. & Ven. 1934) Raatz, 1937 (Photo 2).

Pteridophyta

Psilotaceae

Psilotoidosporites gen.nov.

Typus generis: *Psilotoidosporites salgotarjanensis* (Sics. 1959) comb. nov.

Diagnose: *Azonomonolete* Sporen mit tiefer Laesura, die die Ecken nicht erreicht aber länger als die Hälfte der Sporenlänge ist. Exine zweischichtig mit rugulater Skulptur.

Bemerkung: Die Sporen sind weder mit Foveen noch mit Warzen oder Netzelementen geschmückt, sie weichen mit ihrer rugulaten Skulptur von den Gattungen *Microfoveolatosporis* W. Kr., *Verrucatosporites* Th. & Pf., *Polypodiisporites* R. Pot. *Polypodiidites* Ross ex Couper ab.

3. *Psilotoidosporites salgotarjanensis* (Sics.. 1959) comb. nov. (Photo 3).

Syn.: *Rugulatosporites salgotarjanensis* nov. sp. — Simoncsics, 1959, S. 183. Taf. I, Photo 28.

Diagnose: s. bei *Rugulatosporites salgotarjanensis.*

Selaginellaceae

4. *Muerrigerisporis menkesiensis* sp. nov. (Photo 4)

Holotypus: Exemplar zu Photo 4, Präp.: M-I-3-171 (37,2-108,7). Botanisches Institut der Univ. Szeged. Locus typicus: Kohlengebiet in Nógrád, Flöz Ménkes I. Stratum typicum: Braunkohlenflöz, Braunkohle; Miozän, Helvet.

Diagnose: Formspecies der Gattung *Muerrigerisporis* W. Kr. E-Kontur abgerundetes Dreieck. Cingulum (Dicke bis 5 μm) mit echinaten, stumpfechinaten, ausnahmsweise mit clavaten Ansätzen geschmückt, die Zierelemente sind 1—3 μm hoch mit 1—2,5 μm Basen. Die Proximalseite des Sporenkörpers hat feinere, die distale gröbere verrucat-gemmate Skulptur. Laesuren der Y-Marke geradlinig, lang, reichen jedoch nicht bis zum Cingulum, r=3/4—4/5. Maximalgröße der Spore: 37 μm.

Bemerkungen: Die neue Art steht der *M. gracilis* W. Kr. am nächsten. Davon weicht sie hauptsächlich in der Ornamentation ab. *M. gracilis* kommt im Untermiozän nur sehr selten vor. *M. menkesiensis* ist mit ihrem sehr seltenen Vorkommen im Helvet die jüngste Art der Gattung.

5. *Echinatisporis* W. Kr. sp. *A* (Photo 5).

6. *Echinatisporis* W. Kr. sp. *B* (Photo 6).
7. *Echinatisporis* W. Kr. sp. *C* (Photo 7).
8. cf. *Echinatisporis* W. Kr. sp. *D* (Photo 8).

? *Equisetaceae*

9. *Perinosporites sphaericus* Sics. 1964 (Photo 9).

Osmundaceae

Osmunda

10. *Baculatisporites primarius* (Wolff 1934) Th. & Pf. 1953 (Photo 10).
11. *Baculatisporites quintus* (Th. & Pf. 1953) W. Kr. 1967 (Photo 11).

cf. *Todea*

12. *Baculatisporites* Th. & Pf. 1953 sp. *A* (Photo 12).
13. *Baculatisporites* Th. & Pf. 1953 sp. *B* (Photo 13).

Schizaeaceae

Lygodium

14. *Leiotriletes adriennis* (R. Pot. & Gell. 1933) W. Kr. 1959 subsp. *pseudomaximus* (Th. & Pf. 1953) W. Kr. 1959 (Photo 14).

cf. *Lygodium*

15. *Leiotriletes dorogensis* (Kds. 1960) Kds. 1961 (Photo 15).
16. *Leiotriletes dorogensis* (Kds. 1960) Kds. 1961 subsp. *parvus* subsp. nov. (Photo 16).

Holotypus: Exemplar zu Photo 16, Präp.: M-I-10 (30,8-104,5) Botanisches Institut der Univ. Szeged.

Locus typicus: Kohlengebiet in Nógrád, Flöz Ménkes I.

Stratum typicum: Braunkohlenflöz, Kohle mit Pyriteinlagerungen; Miozän, Helvet.

Diagnose: Subsp. von *L. dorogensis*, kleinere als 80 μm. Exine bis 5 μm, trotzdem läßt sich leicht deformieren. Sporenwand glatt-chagrenat; zweischichtig, V=3/1. Laesuren lang, r=2/3—4/5. Maximalgröße des Typenexemplars 58 μm.

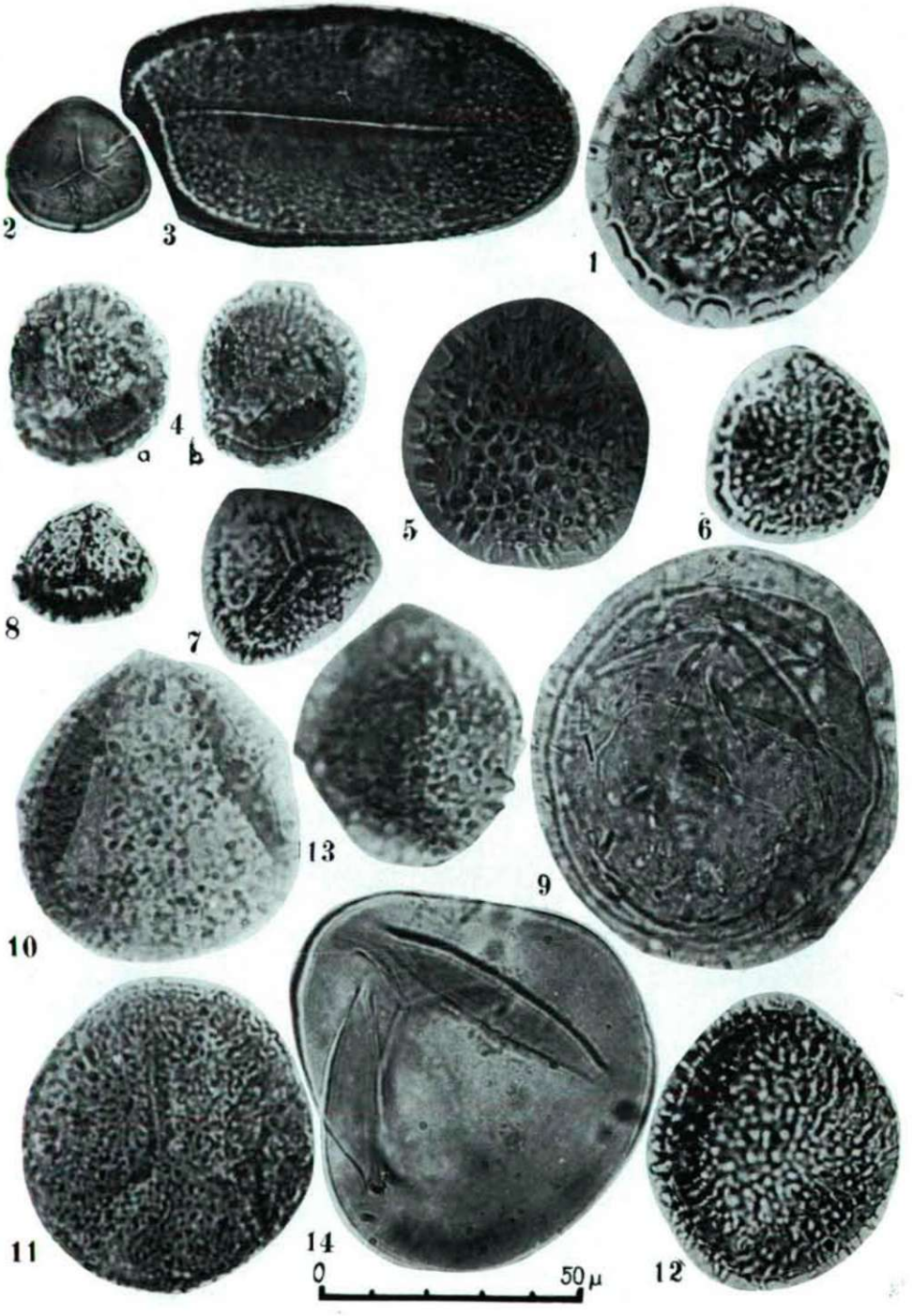
Bemerkungen: Sie kommt hie und da korrodiert und zusammengepreßt in geringem Exemplar vor. Botanisch gehört zu *Schizaeaceae*, cf. *Lygodium* am wahrscheinlichsten.

17. *Leiotriletes microadriennis* W. Kr. 1959 (Photo 17).
18. *Leiotriletes adriennis* (R. Pot. & Gell. 1933) W. Kr. 1959 var. *triplan* Kds. 1961 (Photo 18).
19. *Leiotriletes* (Naumova 1937) R. Pot. & Kremp 1954 sp. var. *triplanoid A* (Photo 19).
20. *Leiotriletes* (Naumova 1937) R. Pot. & Kremp 1954 sp. var. *triplanoid B* (Photo 20).
21. *Trilites multivallatus* (Pflug 1953) W. Kr. 1959 (Photo 21).
22. *Duplosporis* Pflug 1953 sp. (Photo 22a,b).

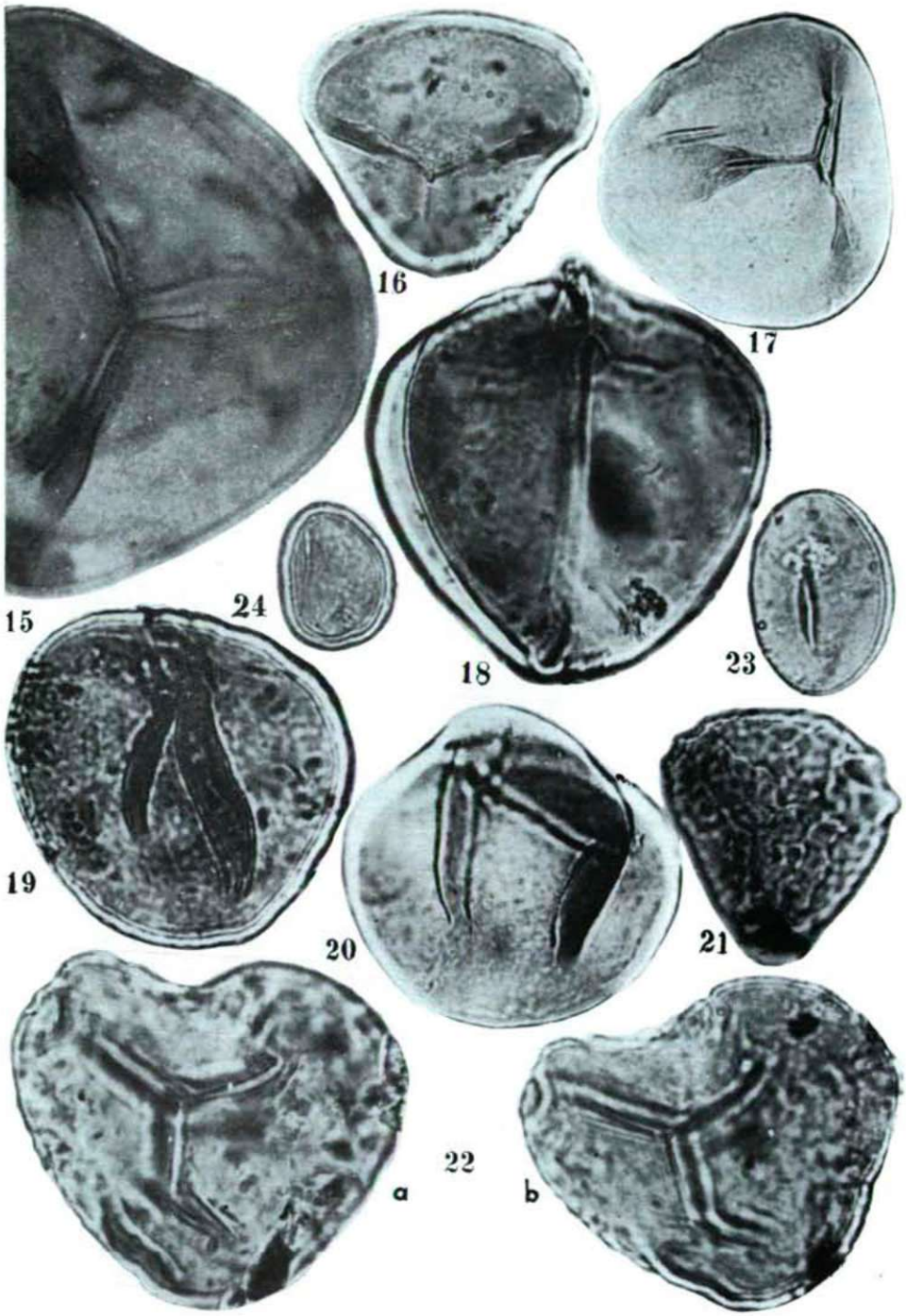
Polypodiaceae

23. *Laevigatosporites haardti* (R. Pot. & Ven. 1934) Th. & Pf. 1953 (Photo 23).
24. *Laevigatosporites minor* (Cookson 1947) W. Kr. 1959 (Photo 24).

TAFEL I



TAFEL II



25. *Laevigatosporites major* (Cookson 1947) W. Kr. 1959 (Photo 25).
26. *Polypodiisporites favus* (R. Pot. 1931) R. Pot. 1934 (Photo 26).
27. *Polypodiidites clatriformis* (Mürr. & Pf. 1952) R. Pot. 1956 (Photo 27).
28. *Verrucatosporites afavus* W. Kr. (Photo 28).
29. *Verrucatosporites alienus* (R. Pot. 1931) Th. & Pf. 1953 (Photo 29).
30. *Baculatisporites nanus* (Wolff 1934) W. Kr. 1959 (? *Pteridium* od. ? *Osmunda*). (Photo 30).
31. *Polypodiaceoisporites megaverrucatus Sics.* 1964 (Photo 31a,b).
32. *Polypodiaceoisporites microverrucatus* Sics. 1964 (Photo 32).
33. *Polypodiaceoisporites* R. Pot. 1956 sp. *A* (Photo 33).
34. cf. *Polypodiaceoisporites* R. Pot. 1956 sp. *B* (Photo 34).

Pteropsida: Sporites incertae sedis

35. *Foveasporis tenuifovearis* Sics. 1964 (Photo 35).
36. *Verrucingulatisporites heteroverrucatus* Sics. 1964 (Photo 36).
37. *Undulatisporites concavus* Kds. 1961 (Photo 37).
38. *Toricingulatisporites margitensis* Sics. 1964 (Photo 38).
39. cf. *Leiotriletes* (Naumova 1937) R. Pot. & Kremp 1954 sp. *A* (Photo 39).
40. cf. *Leiotriletes* (Naumova 1937) R. Pot. & Kremp 1954 sp. *B* (Photo 40).
41. ? *Leiotriletes* (Naumova 1937) R. Pot. & Kremp 1954 sp. *C* (Photo 41).
42. ? *Leiotriletes* (Naumova 1937) R. Pot. & Kremp 1954 sp. *D* (Photo 42).
43. ? *Leiotriletes* (Naumova 1937) R. Pot. & Kremp 1954 sp. *E* (Photo 43).

Gymnospermatophyta

? *Cycadales*

44. *Monocolpopollenites minor* Kds. 1961 (Photo 44a,b).

Ginkgoaceae

Ginkgo

45. *Monocolpopollenites zievelensis* Pf. in Th. & Pf. 1953 (Photo 45.)

Abietaceae

Pinus

46. *Pinuspollenites labdacus* (R. Pot. 1931) Raatz 1937 (Photo 46).
47. *Abietineaepollenites microalatus* (R. Pot. 1931) R. Pot. 1951 (Photo 47a,b).

Picea

48. *Piceaepollenites alatus* R. Pot. 1931 (Photo 48).

Abies

49. *Abiespollenites absolutus* Thierg. in Raatz 1937 (Photo 49).
Keteleeria
50. *Keteleeriapollenites* Nagy 1965 sp. (Photo 50).
Cedrus
51. *Cedripites cedroides* (Th. in Th. & Pf. 1953) R. Pot. 1958 (Photo 51).
cf. *Pseudotsuga* v. cf. *Larix*
52. *Inaperturopollenites magnus* (R. Pot. 1931) Th. & Pf. 1953 (Photo 52).
Taxodiaceae
Taxodium v. *Glyptostrobus*
53. *Taxodiaceaepollenites hiatus* (R. Pot. 1931) Kremp 1949 (Photo 53).
Sequoia v. *Cryptomeria* v. *Metasequoia*
54. *Sequoiapollenites polyformosus* Thiergart 1937 (Photo 54).
Sciadopitys
55. *Sciadopityspollenites serratus* (R. Pot. & Ven. 1934) Raatz 1937 (Photo 55).
Taxodiaceae v. *Cupressaceae*
56. *Inaperturopollenites dubius* (R. Pot. & Ven. 1934) Th. & Pf. 1953 (Photo 56).
? *Taxodiaceae* v. ? *Cupressaceae*
57. *Inaperturopollenites* Pf. & Th. 1953 sp. *A* (Photo 57).
? *Araucariaceae*
58. *Inaperturopollenites intrapunctatus* (Kds. 1961) comb. nov. (Photo 58).
Syn.: *Laevigatasporites intrapunctatus* n.fsp. — Kedves 1961a, p.142, pl. 8, fig 24.

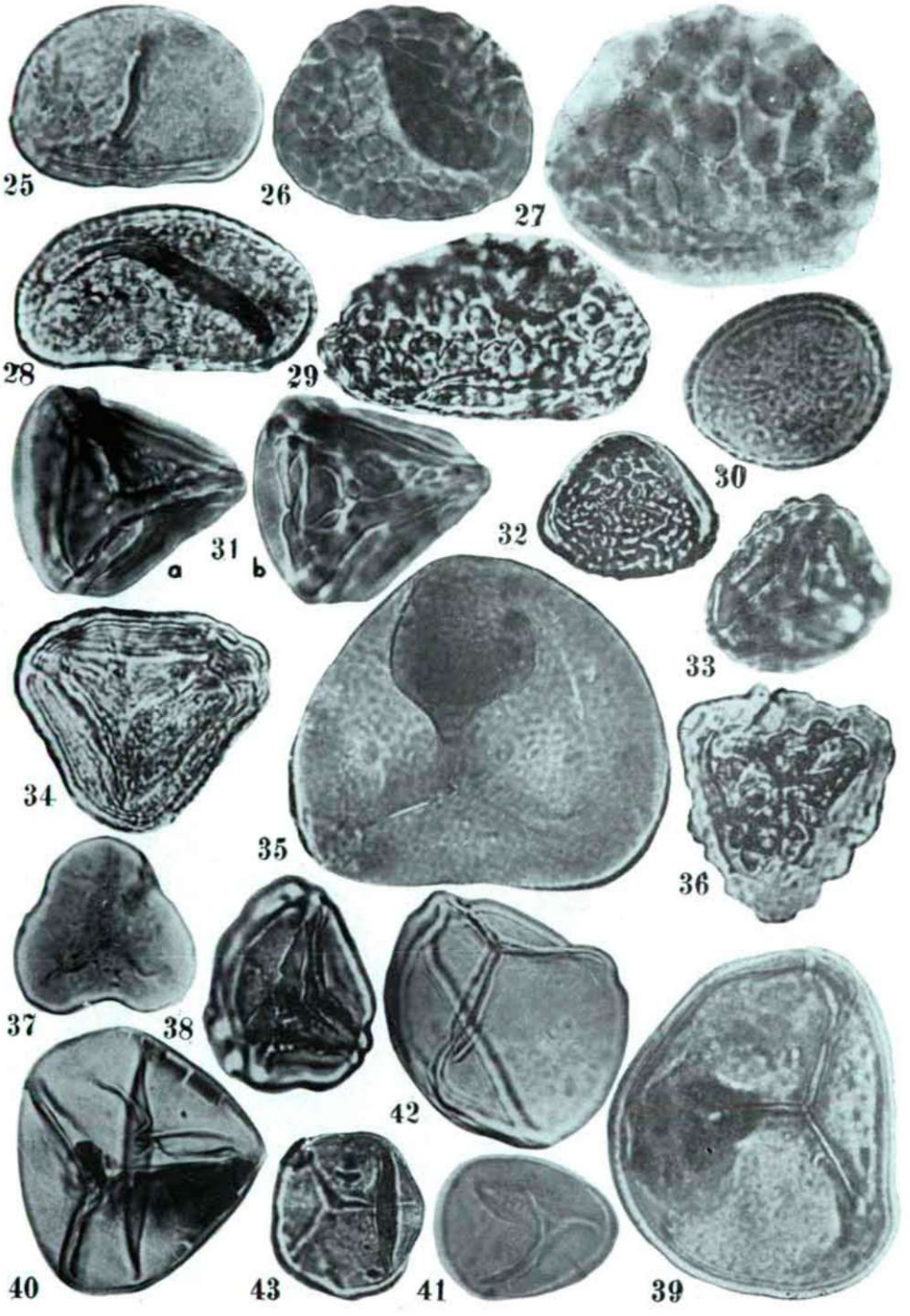
Bemerkung: Es gibt auf dem Pollenkorn kein solches Merkmal, womit es von den *Inaperturopollenites* Pf. & Th. 1953 fgen. abgesondert werden könnte.

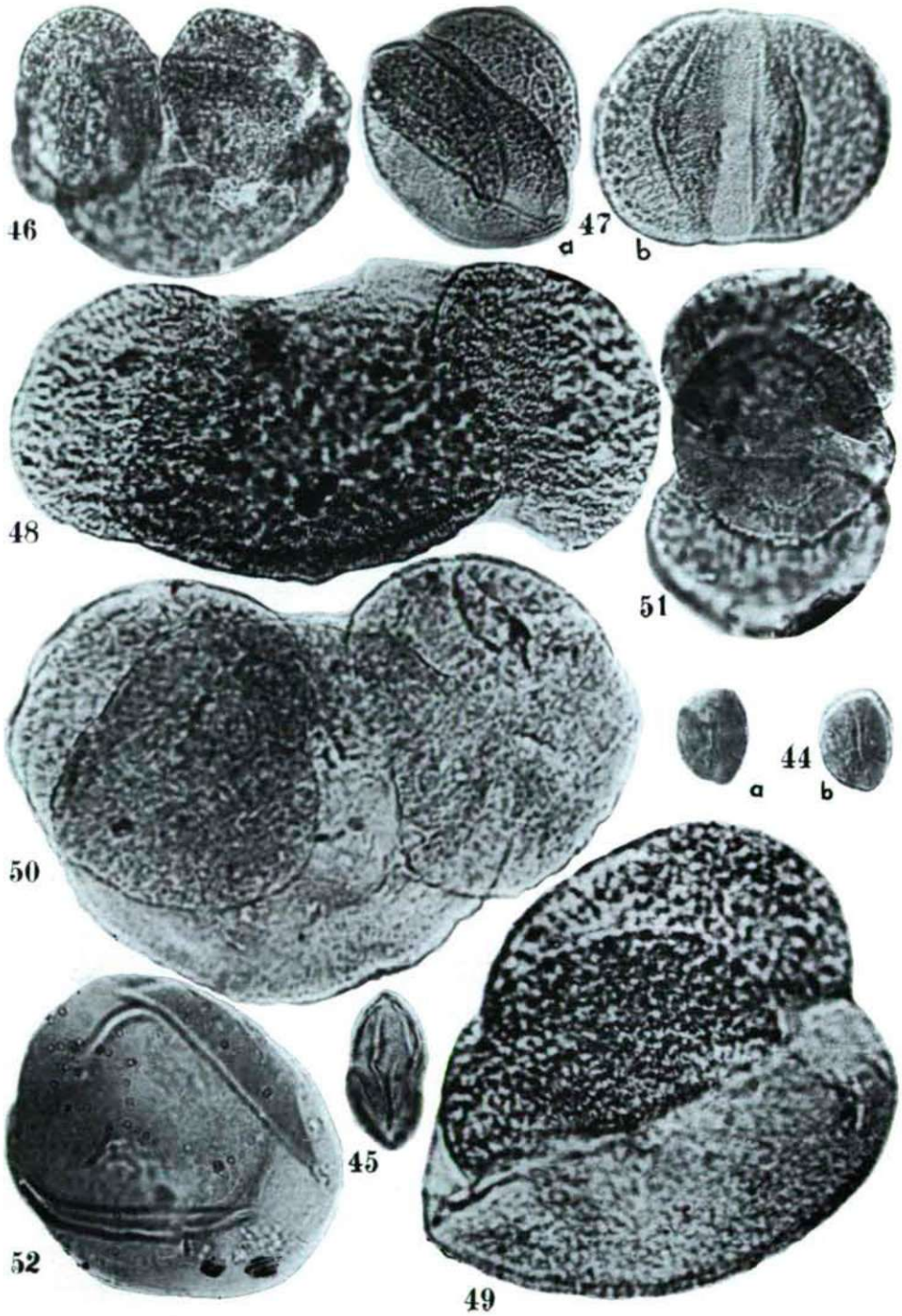
59. cf. *Inaperturopollenites* Pf. & Th. 1953 sp. *B* (Photo 59).
Ephedraceae
Ephedra
60. *Ephedripites (Ephedripites)* W. Kr. 1961 sp. *A* (Photo 60).
61. *Ephedripites (Ephedripites)* W. Kr. 1961. sp. B (Photo 61).

Angiospermatophyta

? *Magnoliaceae*
62. *Monocolpopollenites* Th. & Pf. 1953 sp. *A* (Photo 62).
63. *Monocolpopollenites* Th. & Pf. 1953 sp. *B* (Photo 63).
cf. *Lauraceae*
64. *Inaperturopollenites spinosus* (R. Pot. 1934) comb. nov. (Photo 64).
Syn.: *Poll. spinosus* n.sp. — R. Potonié 1934,S.92, Taf. 5, Fig 18—20,29.
Nymphaeaceae
Nuphar
65. *Nupharopollenites* Nagy 1965 sp. (Photo 65).

TAFEL III

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Hamamelidaceae
Liquidambar

66. *Liquidambarpollenites stigmosus* (R. Pot. 1931) Raatz 1937 (Photo 66a,b).

cf. *Platanaceae*

67. *Platanoidites gertrudae* (R. Pot. 1931) Pot., Thoms. & Thierg. 1950 (Photo 67).

? *Elaeagnaceae* v. ? *Simaroubaceae*

68. *Pentapollenites neogenicus* Sics. 1964 (Photo 68).

Myrtaceae
Myrtus

69. *Myrtaceidites myrtiformis* Sics. 1964 (Photo 69).

Anacardiaceae
cf. *Rhus*

70. *Rhoipites pseudocingulum* (R. Pot. 1931) R. Pot. 1960 (Photo 70).
71. *Rhoipites dolium* (R. Pot. 1931) R. Pot. 1960 (Photo 71).

? *Aceraceae*

72. *Tricolporopollenites striatoides* W. Kr. 1961 (Photo 72).
73. *Tricolpopollenites striatus* Pflug 1959 (Photo 73).

Sapindaceae
cf. *Cupania*

74. *Cupaniedites nogradensis* (Sics 1959) Sics. 1964 (Photo 74).
75. *Cupanieidites* cf. *minimus* W. Kr. 1961 (Photo 75).

Aquifoliaceae
Ilex

76. *Ilexpollenites iliacus* (R. Pot. 1931) Thierg. 1937. (Photo 76).
77. *Ilexpollenites margaritatus* (R. Pot. 1931) Raatz 1937 (Photo 77a,b).

Cyrillaceae

78. *Cyrillaceaepollenites megaexactus* (R. Pot. 1931) R. Pot. 1960 (Photo 78).

Cyrillaceae v. *Clethraceae*

79. *Cyrillaceaepollenites exactus* (R. Pot. 1931). R. Pot. 1960 (Photo 79).

Rhamnaceae

80. *Tricolporopollenites haanradensis* Manten 1958 (Photo 80).

cf. *Rhamnaceae*

81. cf. *Rhamnaceaepollenites insignus* Dokt.-Hrebn. 1957 (Photo 81).

? *Rhamnaceae*

82. ? *Rhamnacidites* (Chitaley 1951) ex R. Pot. 1960. sp. (Photo 82).

Nyssaceae

83. *Nyssapollenites kruschi* (R. Pot. 1934) subsp. *analepticus* (R. Pot. 1934) comb. nov. (Photo 83a,b).
Syn.: *Tricolporopollenites kruschi* (R. Pot.) n.comb. subsp. *analepticus* (R. Pot.) n.comb. — Thomson & Pflug 1953, S. 103—104, Taf. 13, Fig. 14—24.

84. *Nyssapollenites kruschi* (R. Pot. 1934) subsp. *accessorius* (R. Pot. 1934) Pot., Thoms. & Thierg. 1950 (Photo 84).
85. *Nyssapollenites kruschi* (R. Pot. 1934) subsp. *rodderensis* (Thierg. in Pot., Thoms. & Thierg. 1950) comb. nov. (Photo 85).
Syn.: *Tricolporopollenites kruschi* (R. Pot.) n.comb. subsp. *rodderensis* (Thierg.) n.comb. — Thomson & Pflug 1953, S.104, Taf. 13, Fig. 32—33.
86. *Nyssapollenites* Thierg. 1937. asp. *pseudolaesus* (R. Pot. 1931) n. comb. (Photo 86).
Syn.: *Tricolporopollenites kruschi* (R. Pot.) n. comb. asp. *pseudolaesus* (R. Pot.) n.comb. — Thomson & Pflug 1953, S. 104, Taf. 13, Fig. 47—63.

cf. *Cornaceae*

87. *Tricolporopollenites* Pf. & Th. 1953 sp. *A* (Photo 87).

cf. *Cornaceae* v. cf. *Araliaceae*

88. *Tricolporopollenites edmundi* (R. Pot. 1931) Th. & Pf. 1953 (Photo 88).
89. *Tricolporopollenites microeuphorii* Weyl., Pfug & Pantic 1956 (Photo 89).

Araliaceae

90. *Tricolporopollenites wallensenensis* Pf. in Th. & Pf. 1953 (Photo 90).

? *Araliaceae*

91. *Tricolporopollenites* Pf. & Th. 1953 sp. *B* (Photo 91).
92. *Tricolporopollenites* Pf. & Th. 1953 sp. *C* (Photo 92).
93. *Tricolporopollenites* Pf. & Th. 1953 sp. *D* (Photo 93).

Caprifoliaceae

cf. *Sambucus*

94. *Caprifoliipites microreticulatus* (Th. & Pf. 1953) R. Pot. 1960 (Photo 94).

cf. *Caprifoliaceae*

95. *Caprifoliipites Wodehouse* 1933 sp. (Photo 95).

Tiliaceae

Tilia

96. *Tiliaepollenites instructus* (R. Pot. 1931) ex Pot. & Ven. 1934 (Photo 96).

? *Tiliaceae*

97. cf. *Tiliaepollenites* (R. Pot. 1931) ex R. Pot. & Ven. 1934 sp. (Photo 97).

? *Oleaceae*

? *Fraxinus*

98. *Tetracolporopollenites* Pf. & Th. asp. *laesus* (Photo 98).

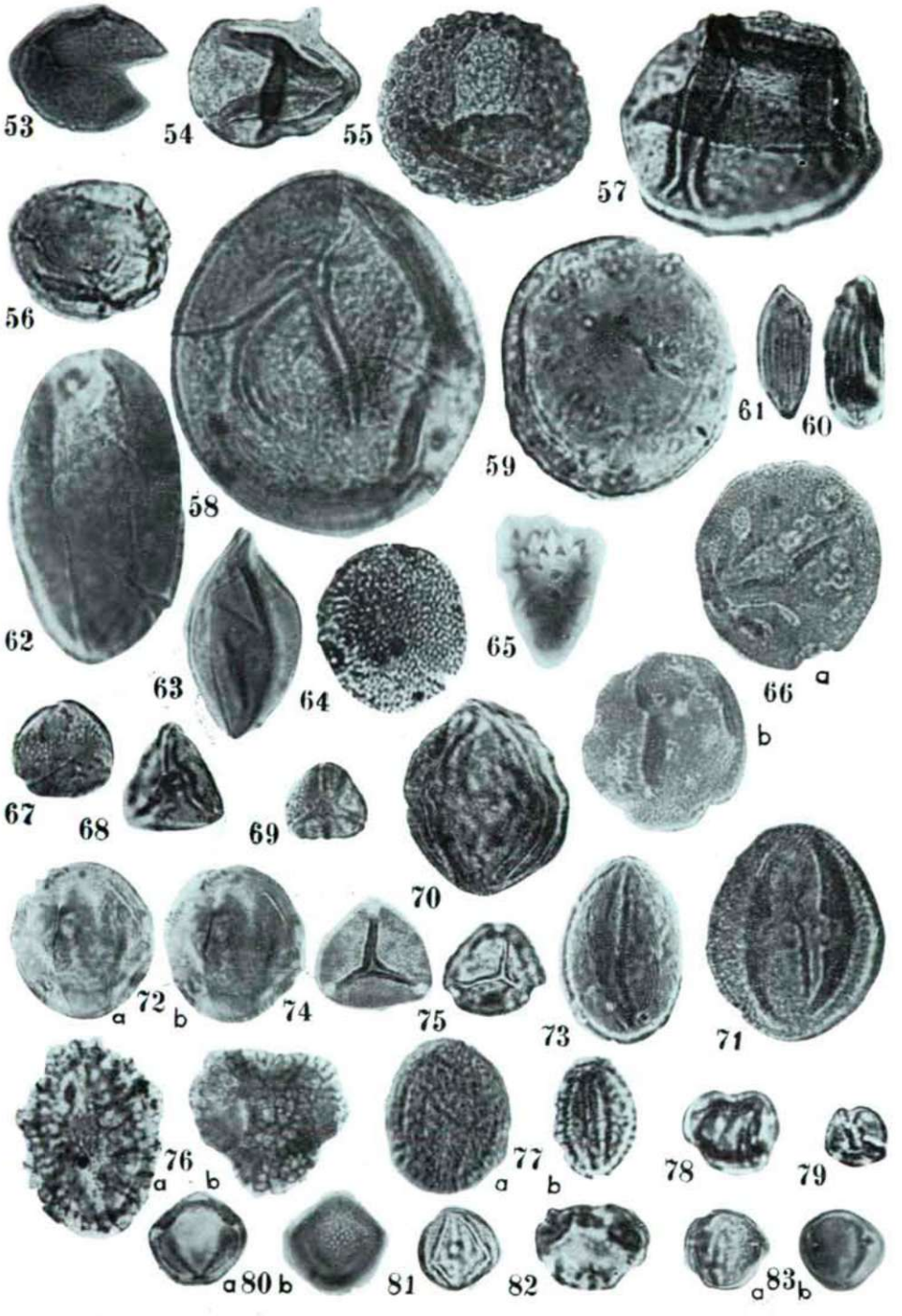
? *Compositae*

99. *Compositoipollenites parvoechinus* Sics. 1964 (Photo 99a,b).

Ericaceae

100. *Ericipites ericius* (R. Pot. 1931) R. Pot. 1960 (Photo 100).
101. *Ericipites callidus* (R. Pot. 1931) comb. nov. (Photo 101).
Syn.: *Pollenites callidus* n.sp. — R. Potonié 1931a, S. 329, Taf. 2, Fig. 24.,27.

TAFEL V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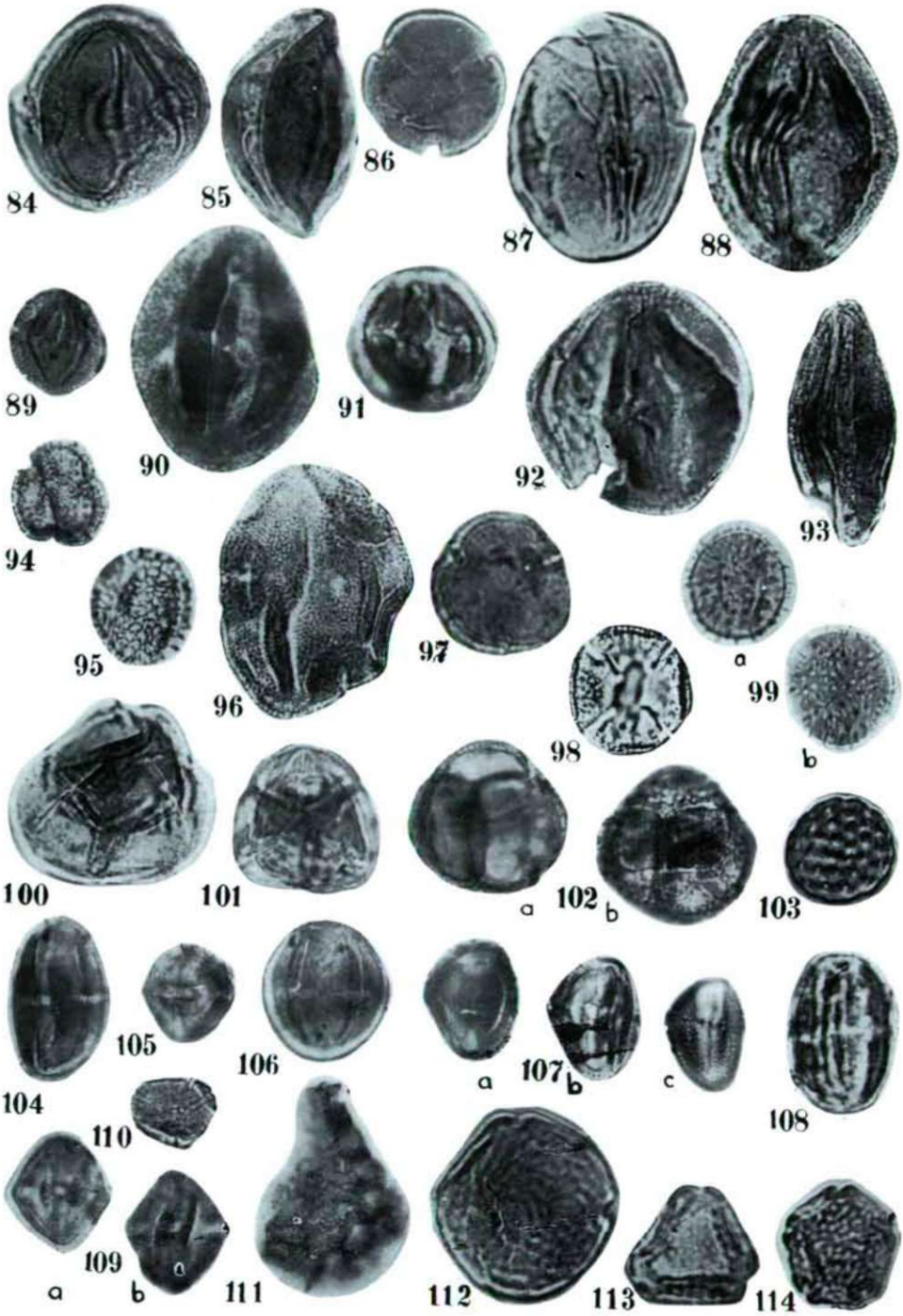
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102. *Ericipites discretus* (R. Pot. 1934) Nagy 1965 (Photo 102a,b).
Chenopodiaceae
103. *Chenopodipollis multiplex* (Weyl. & Pf. 1957) W. Kr. 1966 (Photo 103).
Sapotaceae
104. *Sapotaceoidaepollenites obscurus* (Pf. & Th. 1953) Nagy 1965 (Photo 104).
cf. *Sapotaceae*
105. *Sapotaceoidaepollenites biconus* (Pf. in Th. & Pf. 1953) Nagy 1965 (Photo 105).
106. *Sapotaceoidaepollenites* Pot., Thoms. & Thierg. 1950 sp. *A* (Photo 106).
? *Sapotaceae*
107. *Sapotaceoidaepollenites* Pot., Thoms. & Thierg. 1950 sp. *B* (Photo 107a,b,c).
108. *Sapotaceoidaepollenites* Pot., Thoms. & Thierg. 1950 sp. *C* (Photo 108).
? *Styracaeae*
109. *Tricolporopollenites bipyramidalis* Sics. 1964 (Photo 109a,b).
Symplocaceae
110. *Symplocoipollenites* R. Pot. 1951 sp. (Photo 110).
Polygonaceae
Polygonum
111. *Persicarioipollis* cf. *meuseli* W. Kr. 1962 (Photo 111).
Ulmaceae
Ulmus v. *Zelkova*
112. *Ulmipollenites undulosus* Wolff 1934 (Photo 112).
cf. *Ulmaceae*
113. *Ulmipollenites* cf. *validus* (Pf. in Th. & Pf. 1953) comb. nov. (Photo 113).
Syn.: *Polyporopollenites validus* n.sp. (Pf.) — Thomson & Pflug 1953, S. 91, Taf. 10, Fig. 44—51.
114. *Ulmipollenites* Wolff 1934 sp. (Photo 114).
Betulaceae
Carpinus
115. *Carpinuspollenites carpinoides* (Pf. in Th. & Pf. 1953) Nagy 1965 (Photo 115).
Ostrya
116. *Ostryoipollenites rhenanus* (Th. in Pot., Thoms. & Thierg 1950) R. Pot. 1951 (Photo 116).
Corylus
117. *Triporopollenites coryloides* Pf. in Th. & Pf. 1953 (Photo 117)
Betula
118. *Betulaepollenites betuloides* (Pf. in Th. & Pf. 1953) Nagy 1965 (Photo 118).
119. cf. *Betulaepollenites* Nagy 1965 sp. (Photo 119).
Alnus
120. *Alnipollenites verus* (R. Pot. 1931) ex R. Pot. 1934 (Photo 120).
121. *Alnipollenites metaplasmus* (R. Pot. 1931) R. Pot. 1960 (Photo 121).

cf. *Betulaceae*

122. *Triporopollenites* cf. *robustus* Pf. in Th. & Pf. 1953 (Photo 122).

Fagaceae

Fagus

123. *Faguspollenites verus* Raatz 1937 (Photo 123).

cf. *Castanea*

124. *Tricolporopollenites cingulum* (R. Pot. 1931) subsp. *pusillus* (R. Pot. 1934) Th. & Pf. 1953 (Photo 124).
125. *Tricolporopollenites cingulum* (R. Pot. 1931) subsp. *oviformis* (R. Pot. 1931) Th. & Pf. 1953 (Photo 125).

cf. *Quercus*

126. *Tricolpopollenites asper* Th. & Pf. 1953 (Photo 126).
127. *Quercoidites henrici* (R. Pot. 1931) Pot., Thoms. & Thierg. 1950 (Photo 127).
128. *Quercoidites michrohenrici* (R. Pot. 1931) Pot., Thoms. & Therg. 1950 (Photo 128a,b).

cf. *Fagaceae*

129. *Tricolporopollenites genuinus* (R. Pot. 1934) Th. & Pf. 1953 (Photo 129).
130. *Tricolpopollenites liblarensis* (Th. in Pot., Thoms. & Thierg. 1950) Th. & Pf. 1953 subsp. *liblarensis* (Photo 130a,b).
131. *Tricolpopollenites liblarensis* (Th. in Pot., Thoms. & Thierg. 1950) Th. & Pf. 1953 subsp. *fallax* (R. Pot. 1934) Th. & Pf. 1953 (Photo 131).

? *Fagaceae*

132. *Tricolpopollenites* Pf. & Th. 1953 asp. *laesus* (Photo 132).

Juglandaceae

Juglans

133. *Multiporopollenites maculosus* (R. Pot. 1931) Th. & Pf. 1953 (Photo 133).

Pterocarya

134. *Pterocaryapollenites stellatus* (R. Pot. 1931) Raatz 1937 (Photo 134).

Carya

135. *Caryapollenites simplex* (R. Pot. 1931) Ratz 1937 (Photo 135)

Platycarya

136. *Platycaryapollenites miocenicus* Nagy 1965 (Photo 136).

Engelhardtia

137. *Engelhartioidites microcoryphaeus* (R. Pot. 1931) Pot., Thoms. & Thierg. 1950 (Photo 137a,b).

cf. *Juglandaceae* v. cf. *Myricaceae*

138. *Engelhardtioipollenites punctatus* (R. Pot. 1931) R. Pot. 1960 (Photo 138).

Myricaceae

Myrica

139. *Myricipites rurensis* (Th. & Pf. 1953) Nagy 1965 (Photo 139a,b).


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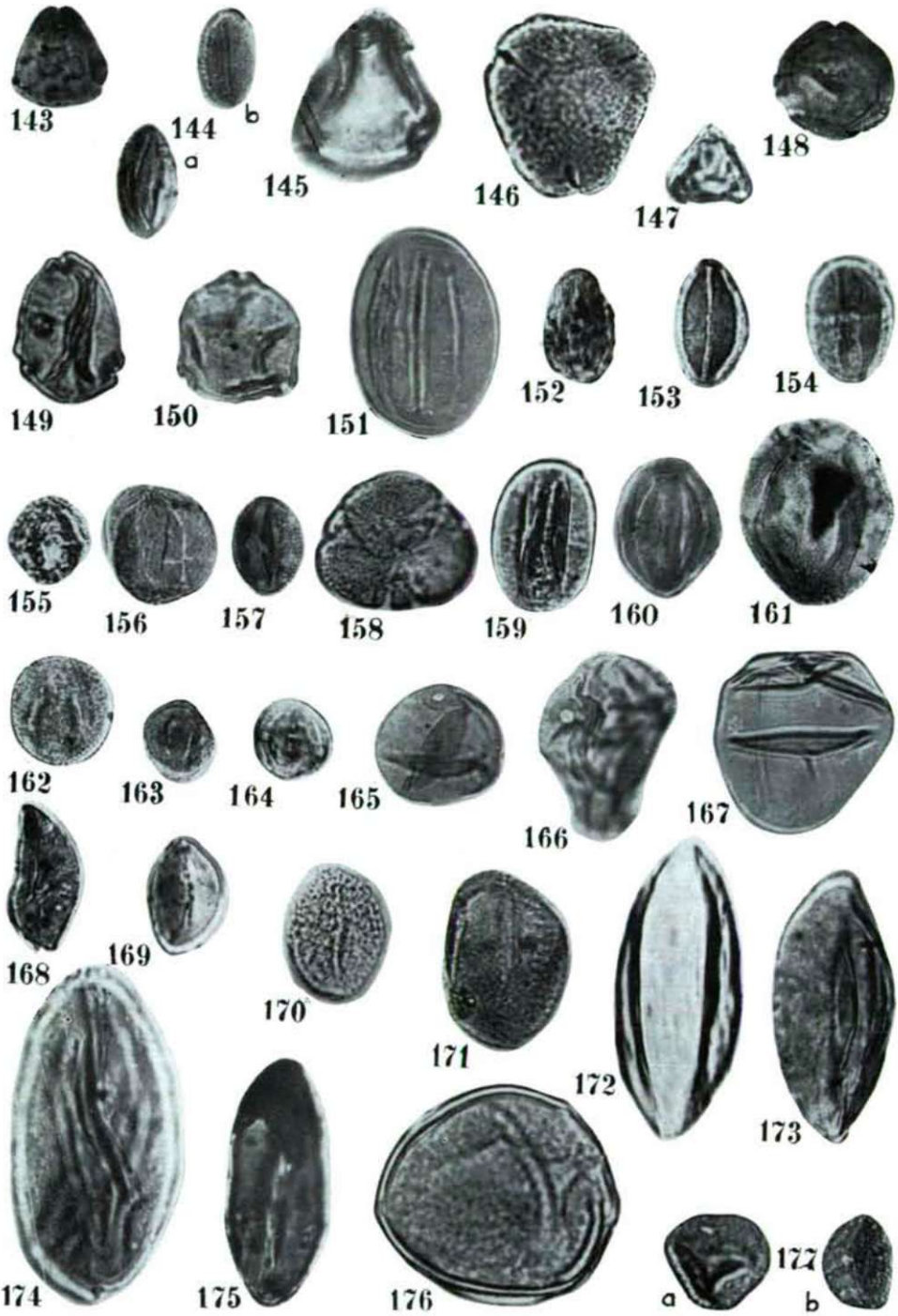
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140. *Myricipites myricoides* (Kremp 1949) Nagy 1965 (Photo 140).
cf. *Myricaceae*
141. *Triatriopollenites bituitus* (R. Pot. 1931) Th. & Pf. 1953 (Photo 141).
142. cf. *Triatriopollenites rurobituitus* Pf. in Th. & Pf. 1953 (Photo 142).
143. *Triatriopollenites quietus* (R. Pot. 1934) Th. & Pf. 1953 (Photo 143).
Salicaceae
Salix
144. *Tricolpopollenites retiformis* Th. & Pf. 1953 (Photo 144a,b).
Dicotyledonopsida:
Pollenites incertae sedis
145. ? *Interporopollenites* Weyl. & Krieger 1953 sp. (Photo 1945).
146. *Trudopollis* Pflug 1953 sp. (Photo 146).
147. *Interpollis* W. Kr. 1960 sp. (Photo 147).
148. ? *Trivestibulopollenites* Th. & Pf. 1953 sp. (Photo 148).
149. ? *Trivestibulopollenites* Th. & Pf. sp. *B* (Photo 149).
150. ? *Triatriopollenites* Th. & Pf. 1953 sp. (Photo 150).
151. *Tricolpopollenites* cf. *parmularius* (R. Pot. 1934) Th. & Pf. 1953 (Photo 151).
152. *Tricolpopollenites* Th. & Pf. 1953. sp. *A* (Photo 152).
153. cf. *Tricolpopollenites* Th. & Pf. 1953 sp. *B* (Photo 153).
154. *Tricolporopollenites cingulum* (R. Pot. 1931) Th. & Pf. 1953 subsp. *fusus* (R. Pot. 1931) Th. & Pf. 1953 (Photo 154).
155. *Tricolporopollenites steinensis* Pf. in Th. & Pf. 1953 (Photo 155).
156. *Tricolporopollenites striatus* Sics. 1964 (Photo 156).
157. *Tricolporopollenites rotundiporus* sp. nov. (Photo 157).
Holotypus: Exemplar zu Photo 157; Präp.: P-III-23 (35,9-96,0), Botanisches Inst. der Univ. Szeged.
Locus typicus: Kohlengebiet in Nógrád, Polyos III.
Stratum typicum: Braunkohlenflöz, Kohlenton, Miozän, Helvet.
Diagnose: Mitglied der Sammelgattung *Tricolporopollenites*. Meridionalkontur zugespitzte Ellipse. Lange Kavernen, enden in Polnähe, am Äquator 2—2,5 μm breit, in der Polnähe schmäler und biegen sich bodenförmig zusammen, bilden kein Cavium. Runde Poren, Porendurchmesser ca. 2 μm. Granulierte Skulptur. Sexine 0,5—1 μm dick, Nexine dünner. Typusgöße 20 $\times$ 15 μm.
Bemerkungen: Der neue Pollentyp unterscheidet sich vom *T. microeuphorii* Weyl., Pflug&Pantic 1958 in den Porenverhältnissen vom *T. microreticulatus* Ph.&Pf. 1953 in Figur und Ornamentation, von den anderen granulat-baculaten Typen in der Größenordnung. Botanische Zugehörigkeit noch unbekannt.
158. ? *Tricolporopollenites* Th. & Pf. 1953 asp. *laesus* (Photo 158).
159. *Tricolporopollenites* Th. & Pf. 1953 sp. *E* (Photo 159).
160. *Tricolporopollenites* Th. & Pf. 1953 sp. *F* (Photo 160).

161. *Tricolporopollenites* Th. & Pf. 1953 sp. *G* (Photo 161).
162. *Tricolporopollenites* Th. & Pf. 1953 sp. *H* (Photo 162).
163. *Tricolporopollenites* Th. & Pf. 1953 sp. *I* (Photo 163).
164. *Tricolporopollenites* Th. & Pf. 1953 sp. *K* (Photo 164).

Gramineae

165. *Graminidites platyanulatus* sp. nov. (Photo 165).
Holotypus: Exemplar zu Photo 165; Präp.: M-I-13-153 (36,7-108,9), Botanisches Inst. der Univ. Szeged.
Locus typicus: Kohlengebiet in Nógrád, Ménkes I.
Stratum typicum: Braunkohlenflöz, Braunkohle, Miozän, Helvet.
Diagnose: Einporiges Pollenkorn. Kugelförmig mit Falten leicht deformiert. Porendurchmesser 2—2,5 μm, Pore mit Anulus von 7 μm Durchmesser umgefangen. Wand ca 0,5 μm, feinkörnig. Typengröße 26 μm.
Bemerkungen: Im Maß unterscheidet er sich von dem größeren G. media Cookson; in der Granulierung von der Gattung *Monoporopollenites* (Meyer 1956) R. Pot. 1960, deren Arten hyalinglatt sind. Der Pollen stammt von der Familie *Gramineae* ab.
166. *Graminidites media* Cookson 1947 (Photo 166).

? *Cyperales*

167. ? *Monoporopollenites* (Meyer 1956) R. Pot. 1960 sp. (Photo 167).

Palmae

168. *Arecipites* cf. *tranquillus* (R. Pot. 1934) Nagy 1965 (Photo 168).
169. *Arecipites scabratus* sp. nov. (Photo 169)
Holotypus: Exemplar zu Photo 169; Präp.: M-I-10 (38,2-121,5), Botanisches Institut der Univ. Szeged.
Locus typicus: Kohlengebiet in Nógrád, Ménkes I.
Stratum typicum: Braunkohlenflöz, Braunkohlen, Miozän, Helvet.
Diagnose: Asymmetrischer monocolpater Pollen. Figur bootförmig. aber eine der Spitzen spitziger, andere stümpfer. Colpus erreicht den *E* und breitet sich dort aus. Exine zweischichtig, V=2/1, bis 1,5 μm dick, chagrenat. Typengröß 24 $\times$ 16 μm.
Bemerkungen: Der Pollen steht nahe dem *A. punctatus* Wodehouse 1933, weicht aber davon darin ab, daß der Colpus der neuen Art sich an den Enden ausbreitet. Unterscheidet sich vom *A. tranquillus* (R. Pot.) Nagy in der Figur, ferner in der Wanddicke. Unterscheidet sich von dem *Monosulcites minimus* Cookson darin, daß dessen Colpus offen und terminal nicht ausgebreitet ist. Der Pollentyp stammt wahrscheinlich von Palmen ab.
170. *Sabalpollenites areolatus* (R. Pot. 1934) R. Pot. 1958 (Photo 170).
171. *Sabalpollenites retareolatus* (Pf. in Th. & Pf. 1953) Nagy 1965 (Photo 171).

cf. *Spadiciflorae*

172. *Monocolpopollenites ingens* Pf. in Th. & Pf. 1953 (Photo 172).
173. *Monocolpopollenites* Th. & Pf. 1953 sp. *C* (Photo 173).
174. *Monocolpopollenites* Th. & Pf. 1953 sp. *D* (Photo 174).
175. *Monocolpopollenites* Th. & Pf. 1953 sp. *E* (Photo 175).
176. ? *Monocolpopollenites* Th. & Pf. 1953. sp. *F* (Photo 176).

Sparganiaceae

cf. *Sparganium*

177. *Sparganiaceaepollenites polygonalis* Thierg. 1937 (Photo 177a,b).

(Fortsetzung und Literaturangaben in den nächsten Heften)

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THE EFFECT OF THE SPECTRAL COMPOSITION OF THE LIGHT ON THE CHLOROPHYLL AND CAROTENOID CONTENTS OF BEAN LEAVES (PHASEOLUS VULGARIS L.)

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Abstract

Experiments under field conditions with perforated coloured polyethylene foils are reported. The amount of the pigments was found to be increased by covering with foils. The amount of the carotenoids decreased with small light intensity in the region of the absorption of carotenoids. The changes of the relative amounts of chromatographically separated chlorophyll-a, chlorophyll-b, α-carotene, α-carotene-monoepoxide and β-carotene-diepoxide, xanthophyll and xanthophyll-epoxide (with some unidentified pigments) are given for three weeks of the vegetation period.

1. Experiments

The experiments were carried out in August and September 1967 under field conditions with coloured polyethylene foils of 0,1 mm thickness. Earlier investigations showed that except the illumination environmental conditions are only slightly influenced by covering with perforated foils (1). During the experiments, in daytime, the intensity of the illumination varied between 10.000 and 50.000 lux.

The spectral distribution of the illumination under the foils was given from the measured spectral distribution of the foils and from the spectral distribution of an average natural illumination (by averageing sunny, cloudy conditions under different heights of the sun). The distribution of the relative intensity is shown in Fig. 1. Here the maximum intensity without foil is arbitrarily taken as unit. From this figure it is seen that the colourless and the blue foils practically cause but a decrease of the intensity (Fig. 1) by 10 and 50 per cent, respectively, whereas under the red foil the spectral distribution of the illumination differs from that under the other foils. Therefore, the application of the colourless foil gives information about the effect of covering; a com-

parison of the results with blue and colourless foils discloses the effect caused by a 50—70 per cent decrease of the intensity of light. Using the red foil the total intensity of light is reduced to 5—7 per cent and 30 per cent in the spectrum region of the absorption of carotenoids and chlorophylls, respectively. The results with red foils, therefore, inform about the case when practically only the chlorophylls are capable to

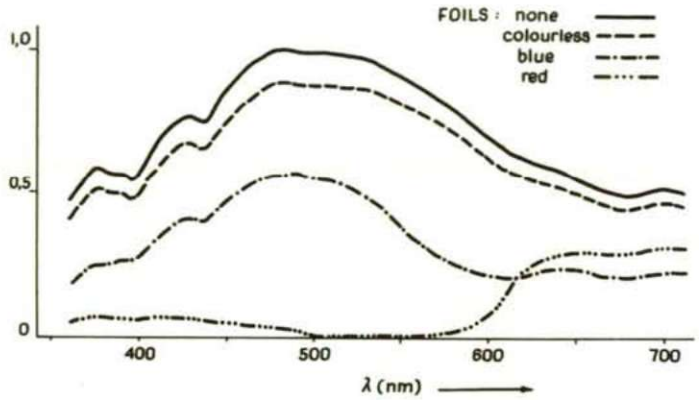


Fig. 1.

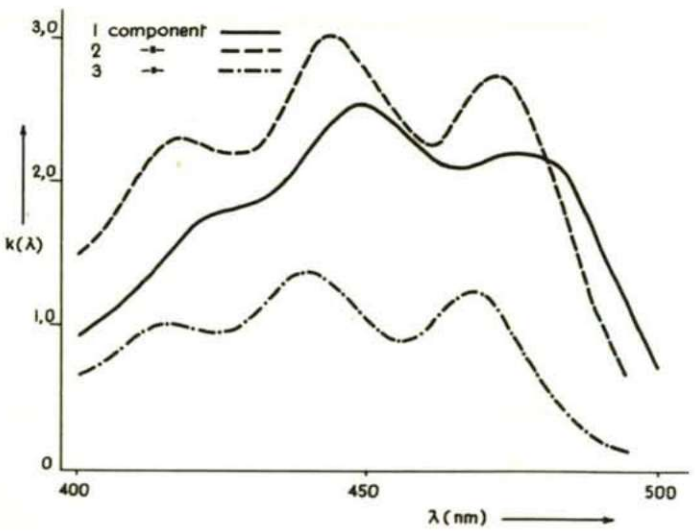


Fig. 2.

energy uptake. A comparison of the results with red and blue foils shows the case when both carotenoids and chlorophylls obtain light energy. (Namely, the spectral distribution of the intensity of light in

the region of the absorption of chlorophylls is practically the same under the blue and the red filters, whereas the blue filter has a considerable transmission in the region of the absorption of carotenoids.)

The leaves were harvested weekly in the same period of the day. 14 g fresh material was homogenized and extracted with petrolether. The components of the pigment system were chromatographically separated with sugar column in the well known manner. The five obtained components (two chlorophylls and three carotenoids) were transferred into ether and the weights were determined from the optical density of the solution. The experiments were done under light protection and at a temperature of 5°C. The criterion of the completeness of the separation of the chlorophylls was to have a ratio of less than 1,33 and 2,82 of the heights of the blue (Soret) and the red maxima for chlorophyll-a and -b, respectively. The absorption spectra of the yellow pigments are shown in Fig. 2 (Fig. 2). The well known chlorophyll spectra are not shown.

2. Results

2.1. The total amount of pigments

Table 1 shows the total amount of the chlorophylls and carotenoids through three weeks in the same units (Table 1). An analysis of the data in this table leads to the following conclusions:

Table 1

Foil	1. week		2. week		3. week	
	Chl.	Car.	Chl.	Car.	Chl.	Car.
none	100	100	90	80	59	83
colourless	98	111	132	103	65	102
blue	104	115	130	112	76	104
red	120	121	122	99	69	86

2.1.1. The amount of pigments is increased by covering.

2.1.2. The amount of pigments is practically not influenced by a 50—60 % decrease of the intensity of illumination.

2.1.3. The amount of the carotenoids compared to that of the chlorophylls decreases if the light intensity is small in the region of the absorption of carotinoids.

2.2. The contribution of the components to the total amount of the pigments

2.2.1. Table 2 exhibits the amount of chlorophyll-a and -b in relative units for the 1—3 weeks with different foils. This table shows that a covering increases the amounts of both chlorophylls (Table 2). The

increase is developed by the 2. and 3. week and especially applies to chlorophyll-b with about 50 per cent. A halving of the illumination does not cause any influence. If only the chlorophylls get light their amount increases in the first week but no increase is found in the further weeks. On the contrary, the amount of chlorophyll-b is considerably reduced by the end of the third week.

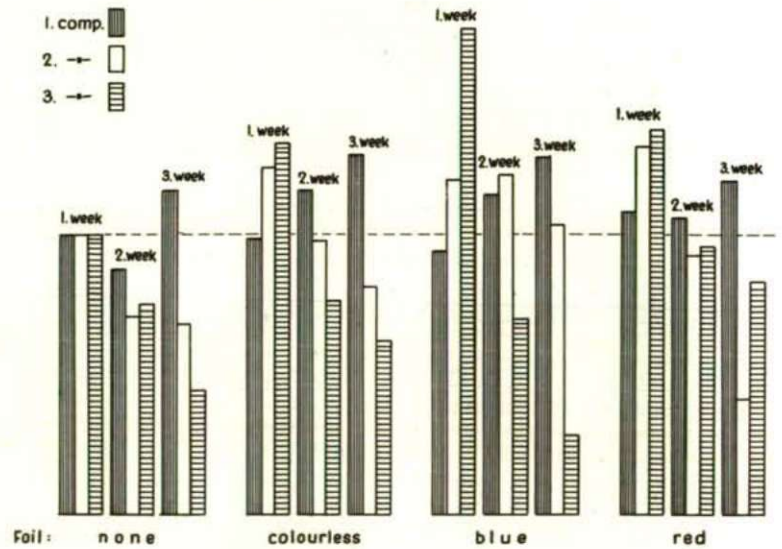
Table 2

Foil	1. week		2. week		3. week	
	Chl-a	Chl-b	Chl-a	Chl-b	Chl-a	Chl-b
none	100	100	88	91	119	102
colourless	98	102	128	128	135	149
blue	105	119	129	127	150	141
red	120	145	120	126	136	121

As for the ratios of the amounts of chlorophyll-a and -b no definite change was found. There is a tendency of increasing of the ratio with the time. A covering and a decrease of the intensity of the illumination by 50—60 per cent show an increased contribution of chlorophyll-b to the ratio.

2.2.2. Carotenoids

Fig. 3 shows the variation of the amount of carotinoids in relative units of the amounts of the first week in all the three components (Fig. 3.) An analysis of the data leads to the following conclusions:



2.2.2.1. The amount of the components increases if the plant is protected with covering by colourless foil. The amount of the 1. component increases with the time as well, whereas the amounts of the 2. and 3. component decrease with time.
2.2.2.2. The decrease of the intensity of illumination by 50—60 per cent does not influence the amount of the 1. component, however, the amount of the 2. component accumulates with the time and the amount of the 3. component highly decreases after a sudden increase in the beginning.
2.2.2.3. If practically only the chlorophylls can access light the amount of the 1. and 2. component highly increases in the first period of time after which a decrease follows. The behaviour of the 3. component is reverse.

2.3. Analysis of the carotenoids

The three components of carotenoids obtained after a single separation with sugar column were identified in the following way.

Component 1. This component is found right below the bands of chlorophyll-a and chlorophyll-b in the sugar column and the maxima of the absorption spectrum of this components are in three different solvents practically at the same wavelengths where the bands of α-carotene are found (see Table 3). Therefore, component 1. is identified as α-carotene.

Table 3

Compound	Solvent	λ_{max} in nm	
		measured	literature/4/
-carotene	hexane	476 ; 449	478 ; 448
	benzene	490 ; 462	492 ; 461
	chloroform	487 ; 460	485 ; 454
-carotene-monoepoxide	hexane	472,2 ; 443,5	478 ; 447
	benzene	487 ; 455	492 ; 460
	chloroform	483 ; 452,5	492 ; 459
-carotene-diepoxide	hexane		470 ; 443
	benzene		485 ; 456
	chloroform		484 ; 456
xanthophyll-epoxide	hexane	465 ; 445	471 ; 442
	benzene	482,5 ; 452	482 ; 453
	chloroform	477,5 ; 449	- -

Component 2. This component seemed to be a mixture of β-carotene-monoepoxide and β-carotene-diepoxide, because it showed a positive hidrochloric acid reaction (3) and the measured maxima of the absorption spectrum in three solvents fitted well into this picture (see Table 3).

Component 3. This component was a mixture of several compounds. The strongly positive hidrochloric reaction and the spectral data mainly show the presence of xanthophyll and xanthophyll-epoxide but the contribution of some other components is not excluded (Table 3.)

This analysis is in accordance with the results of KARRER and coworkers (1948) who reported the presence of α- and β-carotene, xanthophyll and xanthophyll-epoxide in the pigment system of several plants.

In order to draw more detailed conclusions on the physiological role of carotinoids experiments are planned under controlled conditions in phytotrone. With results of better reproducibility the theory of CHOLNOKY and coworkers (1955) could be applied to the changes in the pigment system during the vegetation period. According to this theory primarily carotinoids with ß-ionen rings (ß-carotene and zeaxanthene: 3,3'-dioxy-ß-carotene) are formed in the leaf. On oxygen uptake these will be transformed to epoxides. The epoxides may either be reversibly transformed back to the original compounds with ionen-rings or be transformed to α-isomeres (α-carotene and xantophyll or xantophyll-epoxide).

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RELATIONSHIP BETWEEN PLANT GROWTH REGULATION AND PHOSPHORYLATION PROCESSES II. INFLUENCE OF PLANT GROWTH SUBSTANCES ON METABOLISM OF THE PHOSPHOROUS COMPOUNDS IN PLANT TISSUES

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It is known that the fundamental reactions of hormonal regulation are of genetic level and, apart from that, indole-auxins and several other compounds of hormonal effect have also a direct influence on some metabolic processes. Among these processes, that with the most general importance is the oxidative phosphorylation, i.e., ATP synthesis, the connection of which with hormonal regulation is not even to-day, a question cleared enough.

The influence of auxins, antiauxins, as well that of some phenolic and flavonoid compounds, e.g. the so-called β-inhibitor, upon the change of P/O ratio — in an isolated mitochondria suspension — was studied by a lot of authors (Switzer, 1957; Reid, 1957; Stenlid and Saddik, 1962; Flaig and Schmid, 1962; McDaniel and Sarkissian, 1966; Zhivukhina and Jakushkina 1966). In spite of these investigations, the role of plant growth substances in the oxidative phosphorylation cannot be evaluated in vivo relation, as yet.

The in vivo experiments discussed in this paper have had the aim to follow — by determining the ratio of inorganic phosphorus, nucleotide-polyphosphates and phosphorylated products — in the track of changes in phosphorylation with an indirect method, in plants treated with regulators.

Materials and Methods

For the investigation the materials and methods described in details in Part I of the publication (Sirokmán and Köves, 1968) have been used.

The course of experiments has been as follows.

Isolated plant parts — shoots and roots of pea seedlings or excised leaves — were floated on a nutrient solution containing the required regulator in appropriate concentration. The intact plants were kept in a nutrient culture. After an incubation of 18—24 hours, some samples were taken from the plant material and extracted with ethanol of 70 %. The extract was chromatographed after being

evaporated. The chromatograms were evaluated radiometrically and the total number of impulses of the single compounds, respectively fractions recorded. The compounds were identified with the usual chromatographic methods. For separating ADP and ATP we have cut out the nucleotide spots of the chromatograms prepared in the way described above, and rechromatographed them — on a paper washed with 2 n acetic acid and impregnated with ethylenedliaminetetraacetic acid — in a solvent methanol-ammonia-water 6:1:1. The quantitative determination of ADP and ATP took place on the basis of their UV-absorption observed at a wave length of 260 nm.

Results of experiments

1. Influence of indole-3acetic-acid (IAA) on the incorporation of ^{32}P in alcohol-soluble phosphate compounds of pea seedlings.

According to data of Table 1, the total impulse number of ^{32}P incorporated in pea shoots fed with IAA is lower than that of controls. Expressed in percentage of the total impulse number, the impulse number of the inorganic phosphate fraction is higher, and that of the nucleotide fraction is considerably lower, than those in control plants. As these changes correspond to those expressed by the decrease of P/O, we have investigated under identical conditions, the effect of an uncopuler, 2,4-dinitrophenole (DNP) too, on the alteration of alcohol-soluble phosphate fractions. It appears from Table 1 that the changes induced by DNP in the ratio of fractions compared with one another, are essentially the same as in case of an IAA-treatment; that is to say, the inorganic phosphate ratio increases and that of the nucleotids decreases. The effect of 2,4-DNP is, anyway, considerably greater that that of IAA.

Table 1. Incorporation of ^{32}P in the alcohol-soluble phosphate fractions of pea epicotyl treated with uncoupling and growth regulating compounds. (The data refer to 1 g fresh weight.)

Fraction	Control		IAA 10^{-4} M		2,4-DNP 10^{-4}M	
	C.p.m./ fraction	%	C.p.m./ fraction	%	C.p.m./ fraction	%
Inorg.-^{32}P	12642	43,4	6841	48,2	2105	72,6
Ester-^{32}P	5336	18,3	2653	18,6	427	14,7
"Indole"-^{32}P	444	1,6	594	4,2	-	-
Nucleotide-^{32}P	10754	36,7	4140	29,0	370	12,7
Total	29176		14228		2902	

2. Influence of naturally occurring plant growth substances of phenolic carboxylic-acid character on the incorporation of ^{32}P in the alcohol-soluble phosphate compounds.

The salicylic acid, and some arylhidroxy-cinnamic acid derivates, in a concentration of $10^{-3}M$—$10^{-4}M$, inhibit the elongation as well the auxininduced elongation in several isolated plant organs. The basis of their growth inhibitory effect is — at least partly — the inhibition of the oxidative phosphorylation, as demostrated in vitro by Reid in 1957, by Marinos and Hemberg in 1960, by Stenlid and Saddik in 1962.

The result of our investigations in vivo is shown in Table 2.

Table 2. The radioactivity incorporated in the alcohol-soluble ^{32}P-fractions of pea roots treated with salicylic acid and o-coumaric acid expressed in the percentage of the total activity.

Fraction	$10^{-4}M$ salicylic acid	$10^{-4}M$ o-coumaric acid	control
Inorganic-^{32}P	92,2	95,0	65,2
Ester-^{32}P	3,9	2,4	14,6
"Indole"-^{32}P	1,5	0,8	2,2
Nucleotide-^{32}P	2,4	1,8	18,0

In some ^{32}P-fractions of pea roots, as a result of treatment, the same changes occur like in presence of 2,4-DNP, i.e., activity increased in fractions of the inorganic-^{32}P, but considerably decreased in fraction of the nucleotdie-^{32}P, as compared to the control.

3. Change of the ADP/ATP ratio in the plants treated with $10^{-4}M$ IAA.

Table 3 is demonstrating the amount of ATP, resp. ADP measured by spectrofluorometry in shoots of pea treated with IAA and untreated, further that of the ADP/ATP ratio. According to the data, the total

Table 3. ATP and ADP content of pea shoots treated with IAA.

Sample	µg ATP/g fresh weight	µg ADP/g fresh weight	ADP/ATP
Control	3,0	4,5	1,5
$10^{-4}M$ IAA	1,0	5,5	5,5

amount of the two nucleotides is higher in the control plant, and inside it, the ADP/ATP ratio is increased by IAA-treatment.

4. Incorporation of ^{32}P in the indole compounds of pea shoots in time.

We have established on the basis of earlier radiochromatographic investigations that ^{32}P incorporates in the indole compounds, the F_f-value of which in the chromatograms approaches very much the R_f-values of IAA and tryptophan. Some of them contain a sugar component, as well (Köves and Sirokmán, 1963; 1965; 1966).

Their quantitative change in shoots and roots of seedlings of the intact pea are shown by Figure 1 in the function of time. According to experimental data, the ^{32}P-incorporation is increased by light, both in root and shoot. The curve demonstrating the radioactivity of shoots has a maximum in light, showing that these compounds are incorporated in insoluble compounds and that their incorporation is in light more intensive. (The maximum is considerably smaller in case of measurement results referred to dry matter.)

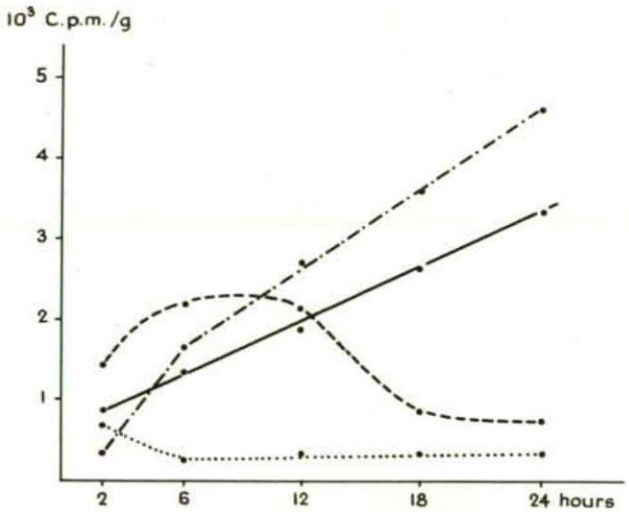

Figure 1. Incorporation of ^{32}P in indole compounds in tissue of pea shoots and roots.

— — — — — — shoot in light
. . . . . shoot in darkness
—.—.—.—.—.—. root in light
——————— root in darkness

The data of Table 4 are showing the influence of regulators applied in the experiments on the degree of the indole compounds in question being phosphorylated. The indices are showing the percentage of the ^{32}P activity incorporated in the indole compounds as compared with the total incorporated activity, in case of the single treatments. It can be ascertained that the salicylic acid and DNP, added into the nutrient

solution, are inhibiting, and IAA is increasing the incorporation of ^{32}P in the indole compounds.

Table 4. Relationship of growth intensity with incorporation of ^{32}P indole compounds in pea epicotyl treated with inhibitors and IAA.

Sample	Elongation in mm	"Indole"-^{32}P acitivity in percentage of the incorporated ^{32}P
10^{-4}M IAA	48	4,2
10^{-4}M salicylic acid	30	1,4
10^{-4}M 2,4-DNP	28	0,0
Control	32	1,6

Discussion of results

The basis of investigating connection between growth regulation and oxidative phosphorylation is the perception of the fact that sytheses accompanying the growth processes do realize only in case of a good energy supply. This has immediately implicated the supposition, that the degree of oxidative phosphorylation i.e., the amount of ATP at disposition, increases by growth-promoting auxins and decreases by compounds of growth-inhibiting effect. But the experimental works carried out on the basis of this supposition have not verified a direct connection like that. For explaining the effect of some growth-inhibiting substances, we can perhaps accept that they uncouple the oxidative phosphorylation. An interpretation of the auxin-effect and of the auxin-synergism is much more complicated.

Marré and Forti (1958) as well as Sen-Gupta and Sen (1961) found an unambiguous connection between oxidative phosphorylation and the growth-promoting effect of IAA. Stenlid and Saddik (1962) demonstrated about several types of regulators, among them compounds of auxin and antiauxin types, as well, that in vitro they decrease the P/O ratio. According to the authors, however, a direct connection is only possible between the effect of antiauxins and the inhibition of oxidative phosphorylation. Flaig and Schmid (1962) found IAA to have an uncoupling effect and explained the growth regulatory and inhibitory effects of auxin with the degree of uncoupling: the oxidative phosphorylation being inhibited in a low degree induces a promotion of growth owing to an increase of the inorganic phosphate level and a better utilization of the intermediary products of metabolism in the syntheses and being inhibited, it induces growth-inhibition owing to the low ATP level.

McDaniel and Sarkissian (1966), as well Zhivukhina and Jakushkina (1966) observed at physiological IAA concentration the increase of the oxidative phosphorylation, and at high IAA concentration its inhibition. In the plants used by them, however, the effect of IAA depended, apart from the concentration, on genotype and endogenous IAA-level, too. Spring and Rowan (1966) demonstrated a decrease of ATP concentration and an increase of the ADP/ATP ratio in plants fed with ^{32}P and treated with IAA; and they found the specific activity of these compounds identical with that of control. Trewavas, Johnston and Crook (1967) demontsrated the increase of the ADP/ATP ratio in case of a 5.10^{-5}M IAA concentration, owing to a decrease of ATP-level. According to their supposition, the decrease is caused by an increase of RNA synthesis induced by auxin.

In agreement with these author's results, it is shown also by our own experiments that in some way, a decrease of ATP level is brought about in the tissue by IAA. Coming up to the expectations, the accumulation of ATP, respectively nucleotide-^{32}P in tissues is decreased in vivo, too, by the salicylic acid known for its uncoupling effect, as well by o-coumaric acid.

We had succeeded, already in the course of our earlier experiments, in demonstrating the presence of some substances in the tissues treated with IAA that contain indole, sugar and phosphate components being similar, therefore, to nucleotides in view of the principle of their structural pattern (Köves and Sirokmán, 1963; 1966). From the fact that their chromatographic behaviour and their UV absorption spectra are almost identical with IAA, respectively tryptophan, the conclusion can be drawn that they are the derivates of these. Their disappearance from the plant tissues as a consequence of 2,4-DNP treatment is showing them being similar to the phosphorous compounds, described but not determined nearer — by Sen-Gupta and Sen (1961), the formation of which is inhibited by DNP. These properties of theirs are showing that they have some role in the phosphorylation processes.

We think so that by establishing the nature and physiological importance of these compounds, the connection between growth regulation and oxidative phosphorylation can be more illuminated. A further question to be cleared is, by what kind of mechanisms the decrease of nucleotides respectively ATP concentration in the plants treated with IAA occurs.

Summary

According to the experiments performed with ^{32}P, in the pea epicotyl treated with IAA the incorporation of ^{32}P in the nucleotides has decreased as compared with control; at the same time, however, the radioactivity of inorganic phosphate has increased. This effect of IAA is similar to that of 2,4-DNP but a little less. The ADP/ATP ratio in pea leaves is increased by IAA.

Growth-inhibiting phenolic compounds inhibit, in a higher degree than IAA, the incorporation of ^{32}P in nucleotides and increase the radio-

activity of inorganic phosphate in tissues.

Among phosphorous products, in different organs of pea seedling indole compounds can be demonstrated, incorporating ^{32}P in their molecules. This incorporation is increased by IAA but decreased by inhibitors.

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DAS PROLIN, DIE DEM WASSERMANGEL DER PFLANZEN ANZEIGENDE AMINOSÄURE

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(Eingegangen am 2. November 1968)

Einleitung

Im Spektrum der freien Aminosäuren der Pflanzen sind auf den Einfluss der Veränderung der ökologischen Faktoren grosse Abweichungen zu verzeichnen. Diese Abweichungen manifestieren sich hauptsächlich im quantitativen Verhältnis der Aminosäuren zueinander: einzelne sind in grösseren, und andere in kleineren Mengen nachweisbar. Die Gesamt-Aminosäurenmenge weist dabei oft Differenzen von 100—300 % auf (Pálfi, 1965; 1968a). Die qualitativen Unterschiede kommen meistens in der Anwesenheit oder dem Fehlen der Amide, insbesondere des Asparagins zum Ausdruck. Aus dem Vorhandensein der Amide kann aber nicht mit Sicherheit auf den physiologischen Zustand der Pflanzen geschlossen werden. Die Amide gelangen nämlich nicht nur im Falle einer guten Nährmittelversorgung bzw. reicher Stickstoffzufuhr, sondern auch infolge ungünstiger Bedingungen der Eiweisssynthese (Phosphormangel, hohe oder niedrige Temperaturen, Licht mangel, hoher Salzgehalt des Bodens, pflanzliche Krankheiten usw.) zur Anreicherung. In den vorliegenden Untersuchungen wird im Aminosäurenspektrum einiger Pflanzen nach einem Indikator gesucht, der typisch bei Wassermangel der Pflanzen in Erscheinung tritt.

Material und Methode

Die Extraktion der bei 65° C fixierten und ausgetrockneten Blätter erfolgte nach Zerreiben mit Quarzsand mit 50 %-igem Ethanol. Nach dem Zentrifugieren wurden die Extrakte mit austeigender ein- und zweidimensionaler Papier- bzw. Dünnschichtchromatographie entwickelt. Solvens war ein Gemisch von Butanol-Eisessig-Wasser (2:1:1) bzw. Phenol-Wasser (4:1). Zur Identifizierung dienten ausser den Rf-Werten Gemische bekannter Aminosäuren. Die Methode wurde von Szalai (1957) und Hartmann (1965) eingehend beschrieben. Bei quantativen Bestimmungen wurde die mit Ninhydrin entwickelten und mit Kupfersalz fixierten Flecke eluiert und spektrophotometrisch ausgewertet. Im Falle des Prolins wurde der blaue Fleck der Isatinreaktion eluiert.

Ergebnisse

Zunächst wurden die Aminosäuren des in Kulturgefässen gezüchteten Weizens (Besostaja 1) studiert. Aus Abbildung 1 geht hervor, dass von den Aminosäuren bei Wassermangel das Prolin die grössten quantitativen Veränderungen aufweist. In den der Trockenheit ausgesetzten Varianten war bereits einen Tag nach Weglassen der eintägigen Wasserdosis der Prolingehalt auf ein Mehrfaches des in den begossenen Kontroll-Varianten registrierten gestiegen. Diesen hohen Prolingehalt behielten die nicht begossenen Varianten bis zum Austrocknen bei. Es ist auch feststellbar, dass die Gesamtmenge der freien Aminosäuren auf die Wirkung des Wasserentzuges erhöht war.

Nun wurde das Aminosäuren-Spektrum der an Wassermangel leidenden *Solanum laciniatum* Ait., Paprika *(Capcicum annuum* L.) und Weizen- (Besostaja 1) Pflanzzen mit den normal mit Wasser versorgten Varianten vergleichend untersucht. An Abbildung 2 ist ersichtlich, dass bei Wassermangel das Prolinniveau bei allen drei Pflanzenarten wesentlich erhöht war. Die Trockenheit hatte den Prolingehalt bei *Solanum laciniatum* auf das Sechsfache, beim Paprika auf das Fünffache und bei Weizen auf das Vierfache des bei den begossenen Kontrollen ansteigen lassen.

Es wurde auch untersucht, welche wesentlicheren veränderungen im Aminosäurenspektrum der von den Trieben isolierten Blätter der drei Pflanzen zustandekommen, wobei sich zeigte, dass auch in der Aminosäurenzusammensetzung der isolierten, anwelkenden Blätter der hohe Anstieg des Prolingehaltes den Hauptcharakterzug darstellt.

Abbildung 3 zeigt einerseits die Aminosäurenzusammensetzung bei den unmittelbar nach dem Abschneiden, und andererseits bei den 1, 3, 5, 9 bzw. 15 Tage nach dem Abschneiden fixierten, gewelkten *Solanum laciniatum*-Blättern. Wie ersichtlich, war die Prolinmenge während des fünftägigen Welkens (auf das Fünffache der Kontrollwerte) gestiegen, um dann 15 Tage fast unverändert zu bleiben. In gleichem Sinne war auch der Gesamt-Aminosäurenspiegel verändert, und zwar in typischer Weise der Asparagingehalt. Die Zusammensetzung der Aminosäuren in den abgeschnittenen, welkenden Blättern erinnert stark an die bei infizierten, kranken Pflanzen (Engelbrecht, 1961; Pálfi, 1964), unterscheidet sich aber davon durch ihre aussergewöhnlich hohe Prolinkonzentration.

Bei Weizen und Gerste ist bereits auch von anderen festgestellt worden, dass bei Wassermangel hohe Prolinkonzentrationen zu verzeichnen sind Coic et al., 1963; Küdrew und Tjankowa, 1966; Barnett und Nayrol, 1966). Aus den Befunden dieser Autoren und unseren eigenen Beobachtungen folgt. dass der primäre schädigende Faktor, der an den Blättern auftritt, der Wassermangel ist.

Singh et al. (1960) wiese nach, dass das meiste Prolin in den alten, absterbenden Blättern zu finden ist; die Prolinanreicherung deutet demnach auf eine Degradation hin. Isotopen-Versuche mit C^{14} (Barnett

und Naylor, 1966) haben gezeigt, dass die Transformation des Prolins bei Wassermangel verzögert ist, seine Synthese aus Glutaminsäure aber unverändert weiterläuft. Wahrscheinlich fungiert das Prolin im Falle von Wassermangel als Reserve-C-N-Quelle.

Unsere Eiweisshydrolysen-Versuche ergaben, dass das viele freie Prolin nicht unmittelbar von Eiweissen abstammt, denn die Zusammensetzung der Eiweisse in den abgeschnittenen, welken Blättern stimmt auch hinsichtlich des Prolins vollkommen mit dem Aminosäurenspektrum der sofort nach dem Abschneiden fixierten Blätter überein. Somit ist das Prolin der welkenden Blätter de novo entstanden. An Hand der Untersuchungen an drei verschiedenen Pflanzenarten konnten wir nachweisen, dass im Falle von Wassermangel unter den freien Aminosäuren der Blätter das Prolin dominiert. Hieraus folgt, dass bei den untersuchten Pflanzen der Prolingehalt hinsichtlich der Wasserversorgung als Indikator des physiologischen Zustandes der Flanzen dienen kann.

Ein grosser Salzgehalt des Giesswassers oder des Bodens kann bei den Pflanzen auch einen Wassermangel verursachen. Wir haben in Kulturgefässen Mais-, Sonnenblumen- und Erbsenpflanzen gezogen und im Alter von 30 Tagen einzig (Varianten) davon 10 Tage lang mit einer stark salzhaltigem Wasser-Lösung (2 % Gesamtsalzgehalt aus Na_2SO_4, NaCl. KCl, $CaCl_2$ $MgSO_4$ und $MgCl_2$) begossen. Bei anderen Varianten wurde durch verringertes Begiessen ein Wassermangel herbeigeführt, während die Kontrollpflanzen auch weiterhin optimal begossen wurden.

Abbildung 4 zeigt ein mit Isatin entwickeltes Chromatogramm, an dem die grossen, dunkelblauen Prolinflecke aufscheinen. Es zeigt sich, das auch das salzige Giesswasser einen starken Wasserdefizit hervorrief, ist doch der Prolingehalt auch hier ausserordentlich gestiegen, ebenso wie bei der wenig begossenen Variante. Bei den optimal begossenen Kontrollen erschien Prolin nur in Spuren.

Bei insgesamt acht gezüchteten Pflanzenarten haben wir den Einfluss des Wassermangels auf die Zusammensetzung der freien Aminosäuren studiert. Abbildung 5 veranschaulicht einige davon. Die grössten und intensivsten Flecke im Chromatogramm zeigt das Prolin. Das Chromatogramm lässt auch feststellen, dass der Wassermangel einen Anstieg des Prolins auf das 20—50-fache veranlassen kann. Diese Erscheinung äusserte sich in sämtlichen Entwicklungsphasen der untersuchten Pflanzen.

Wie aus Abb. 1, 2, 4 und 5 ersichtlich, erschien bei Weizen-, Erbsen- und Tabakpflanzen auf die Wirkung des Wasserdefizits auch die Pipecolinsäure. Wir teilten bereits mit (Pálfi, 1968a; Pálfi und Dézsi, 1968), dass die Pipecolinsäure der Indikator des geschwächten physiologischen Zustandes ist und auch (Stewart et al., 1966; Pálfi, 1968b; 1968c), dass die infolge des Wasserdefizits entstandenen grossen Prolinmengen nicht Ergebnis einer Eiweisszersetzung sind, sordern durch Aminierung von Kohlenhydraten entstehen.

Zusammenfassung

Es wird festgestellt, dass der Wassermangel der Pflanzen durch eine hochgradige Vermehrung des Prolins angezeigt ist. Der abnormal grosse Prolingehalt der Blätter tritt auch bei einem hohen Salzgehalt des Giesswassers bzw. des Bodens auf. Die Prolinvermehrung zeigt ausserdem empfindlich auch das Wasserdefizit der von den Trieben isolierten, welkenden Blätter an.

Bei denselben Pflanzen nimmt proportional dem steigenden Wassermangel die Prolinmenge zu, mit dem Aufhören des Wasserdefizits geht auch die hohe Prolinkonzentration auf das normale Niveau zurück, allerdings erst 6—7 Tage nach Beginn der optimalen Wasserversorgung.

Die durch den Wassermangel bedingte Störung der Eiweiss-synthese zeigt unter den freien Aminosäuren fallweise auch das Erscheinen der Pipecolinsäure an.

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Abb. 1. Freie Aminosäuren der Blätter von mit Wasser versorgten und an Wassermangel leidenden Weizenpflanzen. A=Gemisch bekannter Aminosäuren B=Begossene Weizenflanzen C,D,E,F=1, 2, 3 bzw. 4 Tage nicht begossene Weizenpflanzen. 1=Leu; 2=Phe; 3=Val+Met; Pip=Pipecolinsäure; 4=γ-Amb; 5=Tyr; 6=Pro; 7=Ala; 8=Glu+Thr; 9=Ser+Gly; 10=Glu-NH_2+Asp; 11=Asp-NH_2; 12=Arg; 13=Lys; 14=Cys.

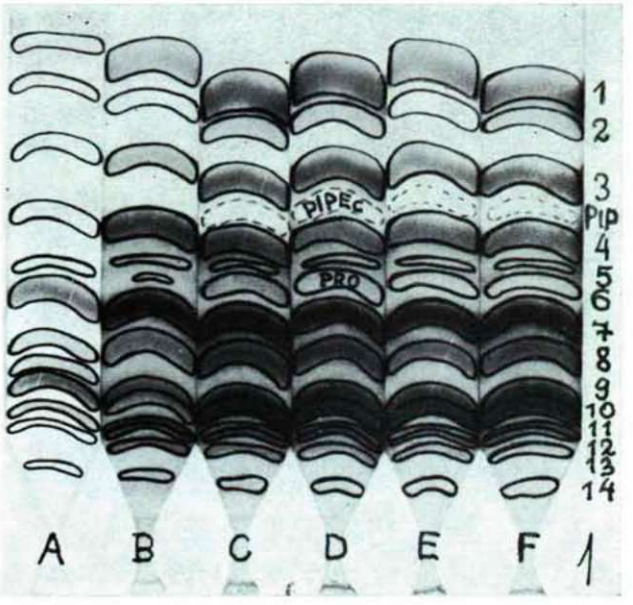
Abb. 2. Freie Aminosäuren der Blätter von mit Wasser versorgten und an Wassermangel leidenden Pflanzen. A=*Solanum laciniatum*, begossen; B=*Solanum laciniatum*, mit Wassermangel; C=Paprika, begossen; D=Paprika, mit Wassermangel; E=Weizen, begossen; F=Weizen, mit Wassermangel; 1—14= wie in Abbildung 1; Pip=Pipecolinsäure.

Abb. 3. Freie Aminosäuren der unmittelbar nach dem Abschneiden fixierten und der nicht sofort fixierten, welkenden *Solanum laciniatum*-Blätter. A= gleich nach dem Abschneiden fixiert; B=1 Tage nach dem Abschneiden fixiert, welkend; C=3 Tage nach dem Abschneiden fixiert, welkend; D=5 Tage nach dem Abschneiden fixiert, welkend; E=9 Tage nach dem Abschneiden fixiert, welkend; F=15 Tage nach dem Abschneiden fixiert, welkend; 1—14=wie in Abbildung 1.

Abb. 4. Zusammensetzung der Aminosäuren in den Blättern der in mit Salzwasser bzw. mit Leitungswasser begossenen (Kontroll) und nicht begossenen Kulturgefässen gezogenen Pflanzen. Entwicklung mit Isatin. A=Mais, B= Sonnenblumen, C=Erbsen. $Index_1$= mit Salzwasser begossen; $Index_2$= mit Leitungswasser begossen; $Index_3$=nicht begossen; 1—14=wie in Abbildung 1. Pip=Pipecolinsäure.

Abb. 5. Die freien Aminosäuren von an Wassermangel leidenden Pflanzen. A,B, C=ein Gemisch bekannter Aminosäuren mit einem Prolingehalt von 20 (A), 40 (B) und 60 (C) μg.; D=Sonnenblumen; E=Erbsen; F=Tabak; G= *Capsicum annuum* L.; H=Spinat. 1—14=wie in Abbildung 1; Pip=Pipecolinsäure.

PLATE I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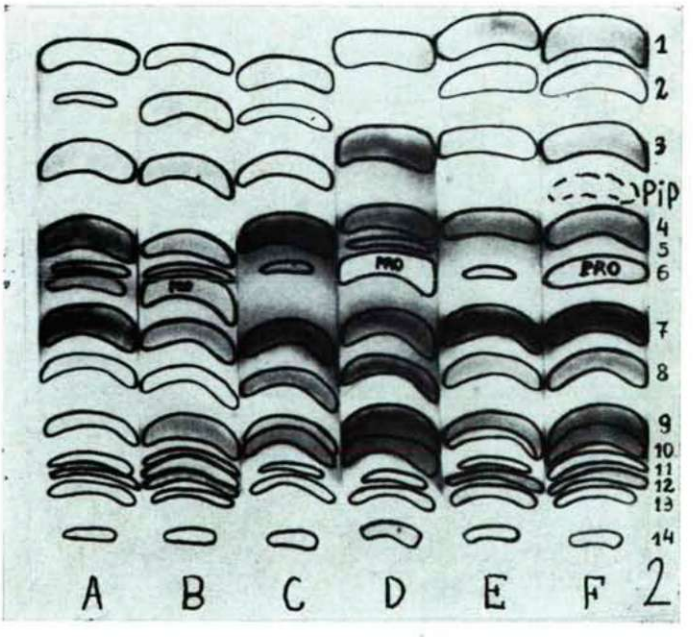
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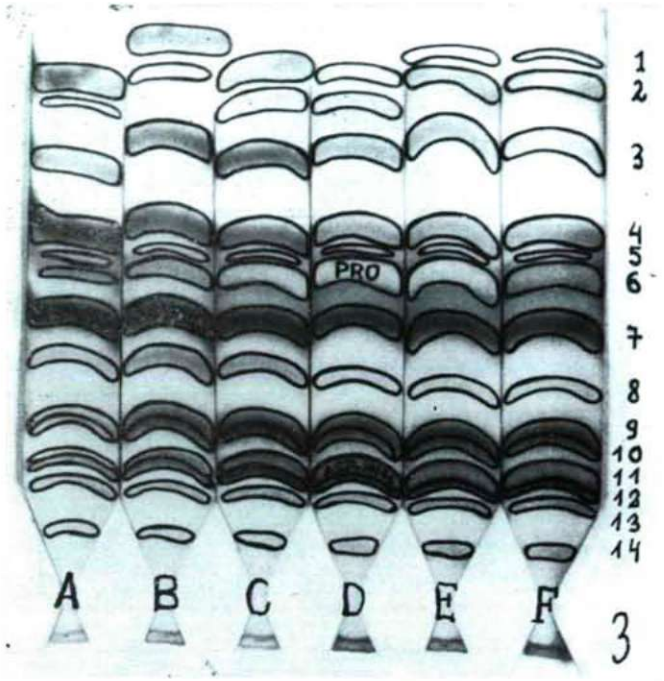
PLATE II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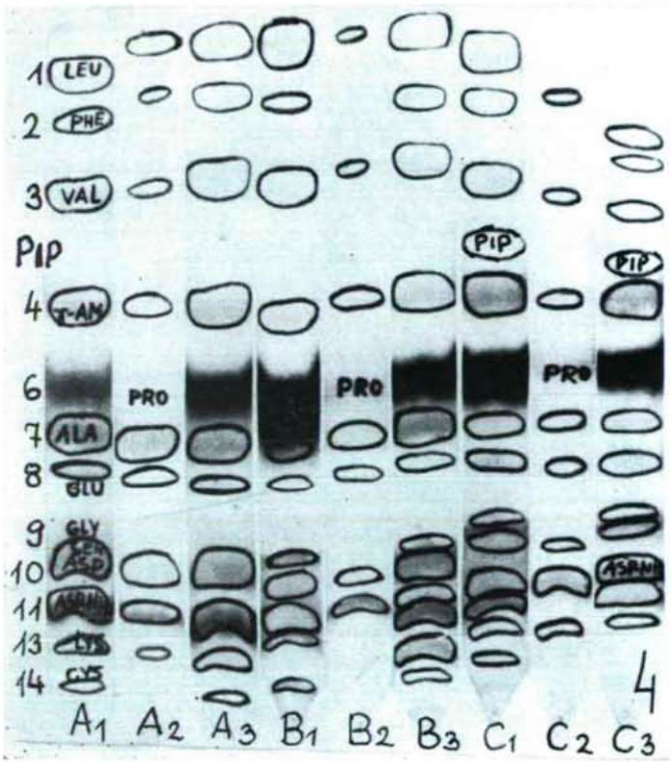
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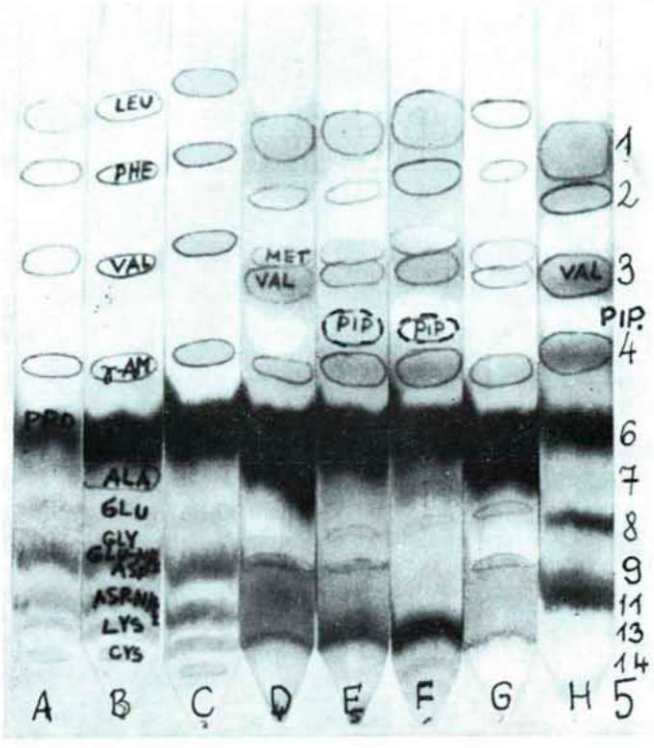
PLATE III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GROWTH INHIBITING ACTIVITY OF SOME STEROID GLYCOALKALOIDS ON HIGHER PLANTS

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The relations between the fungicidal activity and the molecular structure of some steriod glycoalkaloids have recently been established (Ferenczy and Kevei, 1967a,b; Kevei, 1968). The antifungal activity of these compounds is strictly proportional to their ability of complex formation with ergosterol. Their primary acting site is the ergosterol-containing cell membrane system. The release of compounds of low molecular weight (e. g. amino acids) is very characteristic.

In the present study we intended to obtain data on the effects of some steroid glyalkaloids of plant origin on higher plants.

Materials and Methods

The compounds used were chromatographically pure and crystalline tomatin, solaradixin, solamargin, solasonin, and β-solamargin. All compounds were isolated from plants by various methods. (Kevei, 1968.) Melting points: tomatin (I) 272—274°C (decomp.), solaradixin (II) 264°C (decomp.), solamargin (III) 301—303°C (decomp.), solasonin (IV) 292—294°C (decomp.) and β-solamargin (V) 225—226°C.

The concentrations generally applied were 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M. Each compound at each concentration was used at two pH-values (5 and 7) using M/300 phosphate buffer.

Preliminary tests were made with four plant species: *Cucumis sativus* "Kínai Kígyó", *Papaver somniferum* "Fertődi kék", *Sinapis alba* and *Solanum laciniatum*. As the four species gave similar responses, detailed work was carried out with *Cucumis sativus*. The seeds were of the 1967 crop.

Seed germination test. — Two-layer filter papers were placed into 9 cm Petri-dishes and wetted with 6 ml of distilled water (control) or with the test solutions at pH 5 and 7. Four replicates, each with 50 seeds were prepared, incubated in the dark at 25°C for 36 hours, then examined for the percentage of germination. At four-hour intervals the results were checked again.

Growth tests. — After germination on filter papers wetted with distilled water, the seedlings having uniform radicles of 15 mm length were selected and transferred in to the test solutions.

Pieces of bobbin net were stretched on 50 ml beakers filled with the solutions. The seedlings (10 in each beaker) were placed on the net with their radicles dipping into the solution. The controls were buffers at pH 5 and 7. The beakers were placed inside basins covered with glass plates to maintain a humid atmosphere.

After 72 hours of dark incubation at 25°C the seedlings were removed, measured, and the differences between the initial and final lengths of roots and hypocotyls were calculated.

Glycoside absorption, translocation and amino acid release. — To study the absorption and translocation solamargin has been chosen owing to its pronounced biological activity and sensitivity to Hansen and Dam reagent (Hansen and Dam, 1957) modified by Vahouny et al (Vahouny, Borja and Weersing, 1963). These experiments were carried out under aseptic conditions to avoid the interference of micro-organisms. The wetted filter papers in Petri-dishes, the glassware and the solutions were sterilized by autoclaving and the seeds were disinfected by 0,5 % bromine water. After germination, the seedlings were cultivated in buffer solution (pH 7) at 25°C for three days, then 40 uniform plants (the total weight of roots corresponded to 55 $\pm$3 mg dry weight) were transferred to 50 ml solamargin solution (10^{-4} M) at the same pH value. Samples were taken from the solution in every second hour, of incubation and analysed for solamargin and amino acids. Also, plant samples were taken in the same periods. The plants were extracted with methanol for 30 minutes under reflux, and the methanolic solutions were concentrated and applied to chromatoplates in various amounts. The quantity of solomargin in the solutions, roots and hypocotyls was estimated by using thin-layer chromatography as previously described (Ferenczy and Kevei, 1967a). Silica gel G (Merck, nach Stahl), activated at 110° C for one hour, was used as adsorbent and methanol-benzene--ammonia (25 %) (10:20:1) as solvent system. Modified Hansen — Dam reagent was applied (100 mg $FeCl_3$ dissolved in 100 ml glacial acetic acid + 100 ml conc. H_2SO_4) as colour reagent.

In some experiments with β-solamargin, the treatment of roots and the quantitative thin-layer chromatography was carried out as in case of solamargin.

The total quantity of amino acids was determined as previously described (Ferenczy and Kevei, 1967a) using 0,1 ml of each solution, applying on chromatographic paper, drying and developing with ninhydrin. The spots were eluted by 50 % methanol and the colour intensity was measured fotometrically at 520 nm. The extinction values were referred to a standard calibration curve made by alanin. The determination of individual amino acids from both solutions and root extracts was carried out with paper chromatography (Kevei, 1968). Butanol — glacial acetic acid-water (2:2:1) was used as solvent system and Schleicher — Schüll 2043b as paper. For the sake of comparison, a mixture of 20 amino acids was run simultaneously.

When translocation of solamargin from leaves to other organs was studied, 0,1 ml solamargin solution (2×10^{-3}M, pH 7 containing 0,1 % Tween 80 and 0,1 % polyethylenglycol) was applied to the upper surface (17,8$\pm$1,3 cm^2) of one leaf per plant and dispersed. Before treatment, *Cucumis plants* were grown in water culture for two weeks, after treatment for 24 hours (one group in light at 6000 lux, and another in the dark) at 25°C, then cut, the identical parts collected and extracted. The extracts were concentrated and analysed as mentioned before.

Statistics. The standard errors were calculated in the usual manner (Cavalli-Sforza, 1965.) Every experiment was carried out in four replicates.

Results and discussion

Seed germination test. The steroid glycoalkaloids used did not inhibit the germination of *Cucumis* seeds even at the highest concentration used (10^{-4} M). There was only a slight retardation (up to 19 %) in germination rate in case of tomatin, solasonin, and solamargin. The germination rate lagged behind that of the control only in the first 36 hours, but four hours later it reached the control value (98 %). The radicles treated with the above mentioned compounds and with β-solamargin were deformed, shorter and thicker than those of the controls.

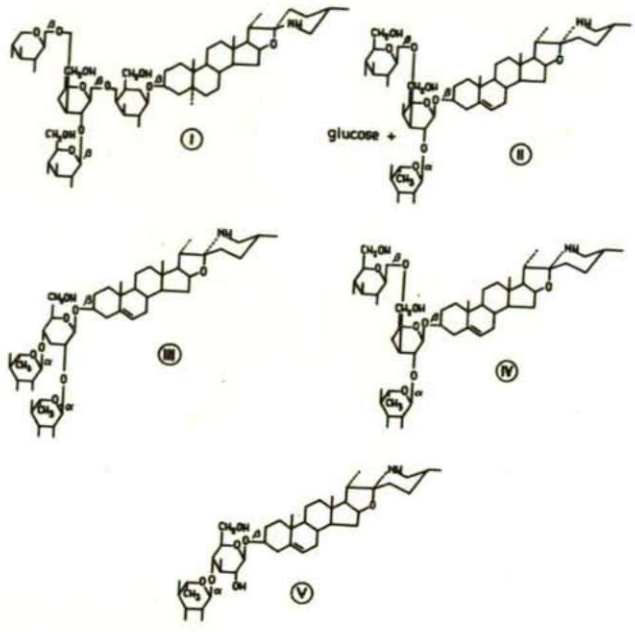

Fig. 1. The comopounds tested. I:tomatin, II:solaradixin, III:solamargin, IV: solasonin, V:β-solamargin.

This morphological aberration was characteristic (Fig. 2).

In an earlier work (K e v e i, 1968) it was also found that filamentous fungi growing in culture media containing the same compounds showed similar features (short, thick and disterted hyphae).

G r o w t h t e s t. — The applied compounds except solaradixin exert a strong inhibitory effect on roots (Fig. 3 and 4). As a consequence, the hypocotyls were also inhibited (Fig. 5 and 6). The inhibition was proportional to the concentrations and was more pronounced at pH 7. In some cases lower concentrations caused slight promotions of the root and hypocotyl growth.

Beside the retardation of growth of the main roots, there occurred also characteristic deformations (Fig. 7).

Comparing these results with those of the earlier antifungal tests (F e r e n c z y and K e v e i, 1967a,b; K e v e i, 1968), it can be established that there is a close similarity in the sequence of activity of the compounds examined. Both on higher plants and on fungi the sequence of activity was as follows: tomatin $\rangle$ solamargin $\rangle$ solasonin $\rangle$ β-solamargin $\rangle$ solaradixin. The activity of β-solamargin is higher in *Cucumis* growth test than in the microbiological tests. The more pronounced activity is not attributable to the alteration of β-solamargin molecule in higher plants as was thought. The quantitative thin-layer

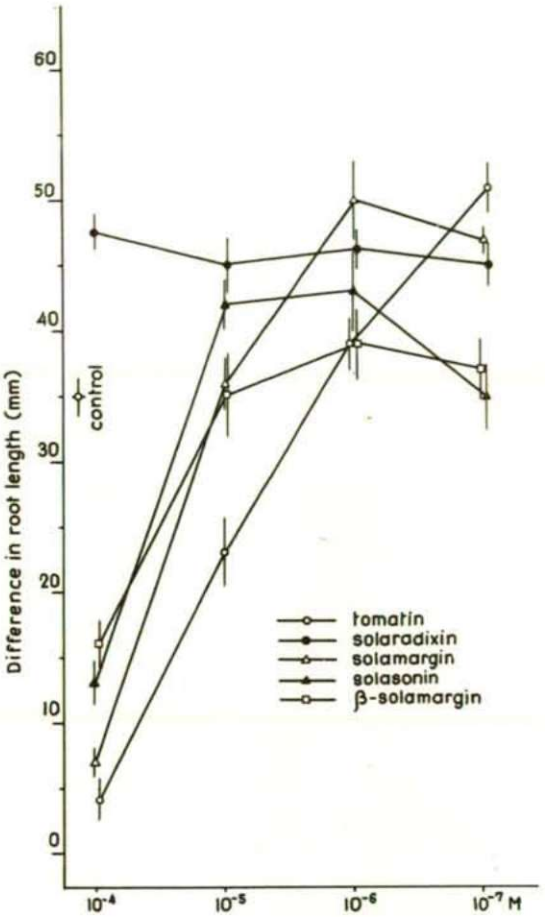

Fig. 3. Growth inhibiting effects on roots of *Cucumis* seedlings at pH 5. Vertical lines represent the standard errors.

chromatography for β-solamargin and for hypothetical derivatives indicated a more intensive absorption of the compound into the roots than into fungi, but no change in the molecular composition. Solaradixin proved to be inactive both in case of higher plants and of microorganisms.

As reported previously, the activity of these compounds is strictly related to the sugar components of the molecules. E.g. the great difference in activity between tomatin and solaradixin (both having four sugar molecules) cannot be attributed to the difference of stereo-position of ring-F or to the absence or presence of double bond in ring-B but to the difference in composition and sequence of the sugar molecules.

Glycoside absorption, translocation and amino acid release. Examining both the solutions and the roots for the amount of solamargin, it was foud that 40 $\pm$ 5% of the quantity of solamargin was absorbed by the roots from the solution. It was also proved that there was no decomposition or other change of the solamargin was absodbed by the rots from the solution. It was also proved that there was no decomposition or other change of the solamargin molecule in plants or in the solution.

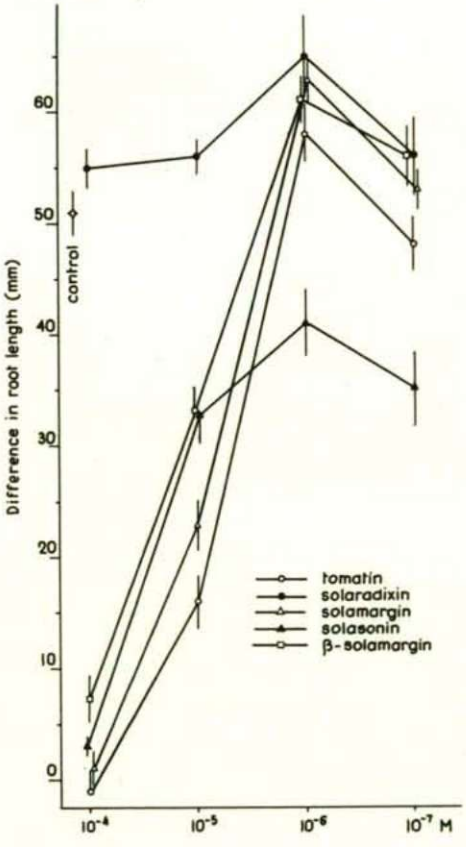

Fig. 4. Growth inhibiting effects on roots of *Cucumis* seedlings at pH 7. Vertical lines represent the standard errors.

The results obtained by chromatography of plant extracts show no translocation of the compound from roots to hypocotyls or from treated leaves to other leaves, stems or roots.

The data of the release of amino acids from roots are shown in Fig. 8. The typical effect of solamargin is the release of amino acids from the roots into the external solution in the first two hours. The subsequent increase in the amount of released amino acids is slight.

It was also found that the release of amino acids was not selective. All kinds of free amino acids to be present in the pool of plant cells were released and their proportion in the external solution was the same as in the cells. The proportoin of amino acid did not show any variation with time.

Concerning the release of amino acids, very similar data were obtained earlier with certain microorganisms too (Ferenczy and

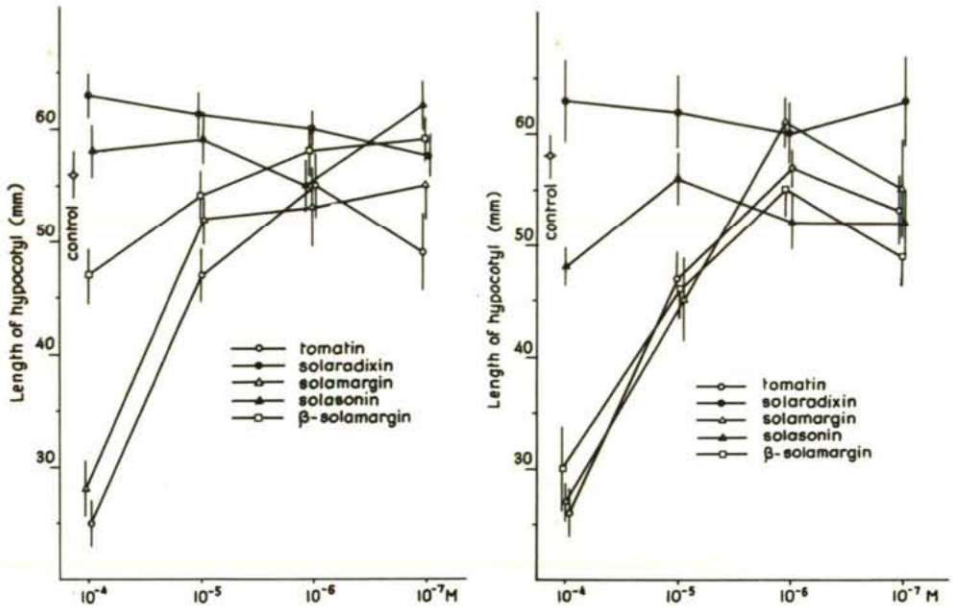

Fig. 5. Growth inhibiting effects on hypocotyls of *Cucumis* seedlings at pH 5. Vertical lines represent the standard errors.

Fig. 6. Growth inhibiting effects on hypocotyls of *Cucumis* seedling at pH 7. Vertical lines represent the standard errors.

Kevei, 1967a,b; Kevei, 1968).

In experiments with various yeast and filamentous fungi it was established that the mode of action of these compounds is the destruction of the ergosterol-containing cell membrane. From the data reported here it may be concluded with fair probability, that the growth inhibiting activity of the steroid glycoalkaloids on roots of higher plants is attributable to the disorganization of the β-sitosterol-containing membrane system.

This research work was carried out during a scholarship granted by the Hungarian Government to one of us (M. R. Rezk) for which he offers his sincere gratitude.

Fig. 2. Distorting effect of tomatin (10^{-4} M); upper row:treated seeds. Lower row:controls.

Fig. 7. Growth inhibiting effect of solamargin at pH 7. Concentrations from left to right:10^{-4},10^{-5},10^{-6},10^{-7} M and control.

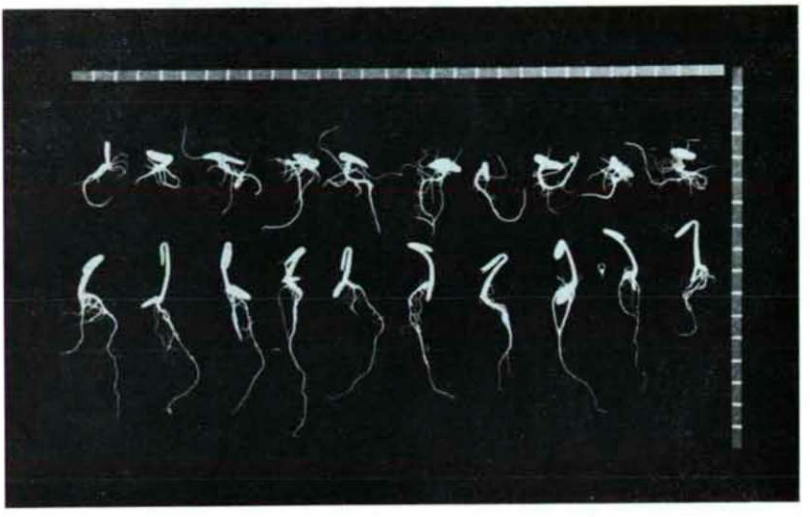

Fig. 2

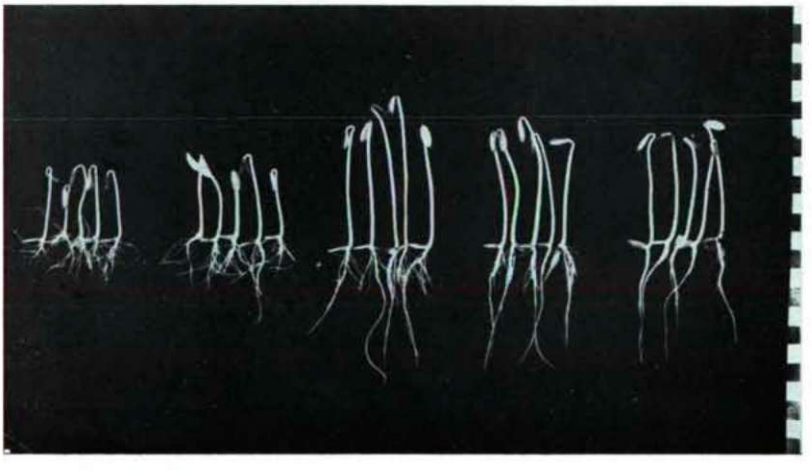

Fig. 7

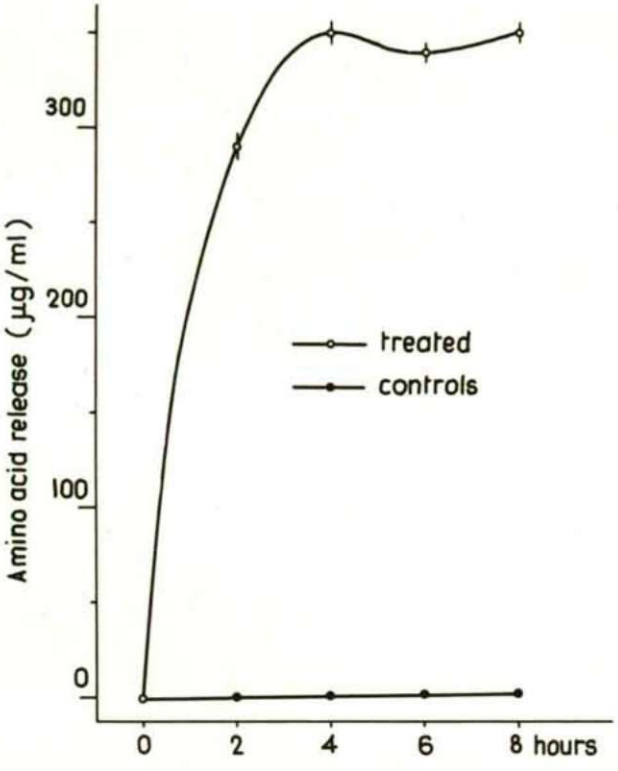

Fig. 8. Effect of solamargin on release of amino acids from roots of *Cucumis* seedlings. Vertical lines represent the standard errors.

Abstract

Five steroid glycoalkaloids (tomatin, solaradixin, solamargin, solasonin, and β-solamargin) isolated from plants were examined for growth inhibiting activity. It was found that these compounds had no inhibiting effect on germnaiton, but four of them caused characteristic root growth inhibition and distortion. There are close relations between the chemical structure and the biological activity. The sequence of activity is: tomatin ⟩ solamargin ⟩ solosonin ⟩ β-solamargin ⟩ solaradixin (inactive).

There was no sign of translocation of solamargin from the roots to the hypocotyls and from the leaves to other organs. This compound caused a quick and non-selective release of amino acids from the root cells. The mode of inhibitory action is probably the disorganization of the sterol-containing cellular membrane system.

These data are in good agreement with the results of earlier work on fungi.

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ELECTRON HISTOCHEMICAL STRUCTURE OF CAPILLARIES IN THE RAT BRAIN

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Introduction

In spite of numerous histochemical and ultrastructural investigations, the structural basis of the blood-brain barrier has not been elucidated fully so far. The investigations performed in the last years (Samorajski and McCloud, 1961; Torack and Barrnett, 1964; Joó and Csillik, 1966; Joó, 1968) called, however, our attention to a possibility that the enzymes demonstrated in the cerebral capillaries play a part in regulation of the permeability conditions of the blood-brain barrier.

Firstly the non-specific alcaline phosphatase activity was described in the brain capillaries (Gömöri, 1941; Landow, Kabat and Newman, 1942). The light microscopic investigations concerning the localization of alcaline phosphatase have yielded contradictory results. According to the investigations of Landow, Kabat and Newman, 1942; Bourne, 1958; Bannister and Romanul, 1963 phosphatase activity can be found in the endothelial cells of brain vessels; according to the results of Leduc and Wislocki (1952) and Wislocki and Dempsey (1948) it is, anyway, lacking in the smaller brain and in the walls of arterioles. Where it can be demonstrated, it is localized to the connective-tissue sheath. Adenosintriphosphatase (ATP-ase) activity of capillaries was described by numerous authors (Torack, Besen and Becker, 1961; McIlwain, 1963). Butyrylcholinesterase (BuChE) activity in brain capillaries was first described by Koelle (1954). This enzyme activity had previously been regarded as a "common diffusion artifact" by Gerebtzoff (1959); later on, however, at his experiments with perfusion he got convinced of it being a real enzyme activity of capillaries. Friede and Fleming (1964) have observed an intensive cholnesterase activity in the cerebellum of 12 species.

In this present paper the fine structural localization of the above mentioned enzymes will be described as demonstrated with histochemical

methods in the brain capillaries, and their possible role will be discussed in the regulation of the permeability of the blood-brain barrier for the macromolecules.

Materials and Methods

Our studies were performed on 20 albino rats weighing 150—200 g. The brains were fixed with immersion, resp. in another case perfusion technique, in the mixture of formaldehyde-glutaraldehyde buffered with 0,14 M sodium cacodylate to pH 7,4 for 4—6 hours (Törő and Joó, 1966). In another case we have carried out a perfusion fixation with a fixing solution buffered with 0,14M sodium cacodylate made freshly according to Karnovsky (1965) from paraformaldehyde. After being fixed, our samples were rinsed in a sodium cacodylate buffer containing 0,44 M sucrose in a temperature of + 4°C, for a night. The histochemical reactions were elicited on 50 μ thick sections prepared with a freezing microtome, resp. on thin cuts made by hand with a razor blade.

For demonstrating the non-specific alcaline phosphatase activity we have used Gömöri's post-coupling method (1939), resp. its modification carried out by Fredricsson (1952). For indicating the ATP-ase activity electron histochemically, we have applied the procedure described by Padycula and Herman (1955). For demonstration of the cholinesterase (ChE) activity we have used Karnovsky's "direct colouring method" (1964) and an electron histochemical modification of the thiocholine — method, elaborated by us earlier, so-called "Pb-thiocholine" method (Joó, Sávay and Csillik, 1965; Kása and Csillik, 1966; Csillik, Joó, Kása and Sávay, 1966).

After carrying out the histochemical reactions — except the "direct colouring method" applied for demonstrating the ChE activity — and a short rinsing, we performed a convertation in a 2 p.c. ammoniumpolysulfide solution neutralized with concentrated acetic acid. The samles were postfixed for 30 minutes, in a 1,33 p.c. osmium solution buffered with s-collidine, on +4°C (Benneth and Luft, 1959), and embedded in "Durcupan" (Fluka) after gradual dehydration with alcohol. Thin sections were made in ultramicrotome of types LKB and Porter-Blum, placed on non-coated (300 mesh) grids. Our preparations have been investigated with a table electron microscope Teesla 242D, without being contrasted or, in another case, after Reynolds's (1963) Pb-cirate contrasting.

Results

We found enzyme activity first of all in the cytoplasm of the endothelial cells of brain capillaries, after we had applied Gömöri's method (1939) for demostrating the non-specific alcaline phosphatase activity. The final product of reaction fills in the cytoplasm in the form of an electron dense precipitate of 400—800 Å in diameter, being present here and there in the subendothelial basement membrane, as well (Table 1.A). In another case, an enzyme activity could be observed in the cytoplasm, mainly localized in the pinocytotic vesicles (Table 1.B). With Gömöri's method modified by Fredricsson (1952), — as used generally for light microscopic investigations — the enzyme activity was found in the field of the endothelial cytoplasm in the form of a rough precipitate (Table 1.C). The electron dense precipitate is localized in the surface membrane of the endothelial cell and in the pinocytotic vesicles of cytoplasm. In the basement membrane, there can sometimes be found some precipitate of much weaker density which is, supposedly, an artefact produced in the course of the histochemical reactions.

The adenosintriphosphatase activity was found in the basement membranes of brain capillaries, as we used the post-coupling

method described by Padycula and Herman (1955). The enzyme activity takes place continuously tracing out well the course of basement membrane (Table 2.A,B). In the cytoplasm of the endothelial cell we have not found any enzyme acivity either in the mitochondria or in the pinocytotic vesicles. In the surface membrane of astrocyte end-feet that surround the capillary we have observed ATP-ase activity, as well.

At using the "direct colouring" method, we have found cholinesterase activity with butyrylthiocholineiodide substrate in the capillary wall (Table 3). The final product of reaction can be observed seriatim in the basement membrane, in the form of an electron dense precipitate 800—1500 Å in diameter. In the cytoplasm of endothelial cell with a butyrylthiocholineiodide substrate we have not observed any enzyme activity. Applying, however, acetylthiocholineiodide as a substrate, we have found a ChE activity both in the cytoplasm and in the basement membrane of the endothelial cell. The end-product of reaction can be observed both in the pinocytotic vesicles (Table 4.A) and in the basement membranes (Table 4.B,C). The final product of reaction is indicating the place of acetylcholinesterase activity in a characteristic way and localized seriatim, even with an acetylthiocholineiodide substrate, in the form of an electron dense precipitate of 100—1500 Å diameter.

Applying methods of "Pb-thiocholine", we have found cholinesterase activity in the cytoplasm of endothelial cell, with butyrylthiocholineiodide substrate (Table 5.A). Electron dense precipitate that would refer to a final product of reaction was not found in other parts of capillary, so not in the glial end-feet close to the basement membrane, either (Table 5.B). Butyrylcholinesterase activity was not found in the field of pericytes, either (Table 5.C). In case of an optimal incubation time, enzyme activity can be observed only in the pinocytotic vesicles (Table 5.D). After performing the procedure for indicating the acetylcholinesterase activity, we have not got any final production of reaction in the cytoplasm of endothelial cells, in connection with a structural element. The electron dense precipitate in preparates like these (Table 6.A,B) took place in basement membrane of the capillary wall. With acetylthiocholineiodide susbstrate, first of all in case of a longer incubation, we have found an enzyme activity also bound to the surface membrane of cytoplasm of endothelial cells (Table 6.C)

We want to mention concerning the localization of ChE activity in the pinocytotic vesicles that, in the course of our investigations, we have found both vesicles showing a butyrylcholinesterase activity and those showing no enzyme activity. We do consider as imaginable that in the endothelial cell not every pinocytotic vesicle has BuChE activity; it seems, however, to be more probable that the pinocytotic vesicles that do not show any enzyme activity, develop during the electron histochemical procedure. As it is known, it is demonstrated by Reale and Luciano (1964) about osmium fixation after the application of the histochemical method, and Kalimo and co-workers (1968) about contrasting with Pb-citrate that they can solve a part of the final production of reaction containing heavy metal, being thus capable of resulting in a "false negative" reaction.

Discussion

The results of investigations in the last years are referring to that the enzymes demonstrated in the brain vessels with histochemical methods can play a role in regulating the permeability relations of the blood-brain barrier. Samorajsky and McCloud (1961) investigating the connection between the phosphomonoesterase activity of cerebral capillaries and the permeability state of cerebral vessels have found so that under experimental conditions (brain oedema, meningeoma, meningo-encephalomyelitis, etc.) where the permeability of the hematoencephalic barrier increases, at the same time, in a characteristic way, also the alcaline phosphatase activity of brain vessels intensifies. Alcaline phosphatase activity, according to the results of our electron histochemical investigations, is localized in the cytoplasm of the endothelial cell. The localization of alcaline phosphatase activity in the pinocytotic vesicles could be well observed particularly if a short incubation time was used. The ultrastructural localization of the enzyme is in harmony with several earlier observations concerning the connection between the alcaline phosphatase activity and the transport phenomena in the capillary and the energy supply of that process (Landow, Kabat and Newman, 1942; Wislocki and Dempsey, 1948; Bourne, 1958; Nandy and Bourne, 1963).

According to the observation of Torack and Barrnett (1964), the adenosintriphosphatase activity is localized in the basement membrane and the surface membrane of glial end-feet in capillaries of brain fields where the blood-brain barrier is functioning. On the other hand, in the cerebral structures that are not defended by the blood-brain barrier, as well in the capillaries in other tissues, the ATP-ase activity can be demonstrated with electron histochemical method in the pinocytotic vesicles of the cytoplasm of endothelial cell (Marchesi and Barrnett, 1964). According to the electron histochemical data of Rechardt and Kokko (1967), however, the ATP-ase activity can be found in the capillaries of the medulla oblongata of rats, like a coarse final product, both in the pinocytotic vesicles of endothelial cells and in the basement membrane. According to Hoff's re-investigation (1968), in the intracranial vessels of rabbits an enzyme activity can only be observed if adenosintriphosphate or adenosindiphosphate is used as a substrate. The main part of the final product of reaction localized in the basement membrane from the elements of the capillary wall. According to our own investigations, using the method described by Padycula and Herman (1955), as well, in the brains of rats the ATP-ase activity is localized in the basement membrane of the endothelial cell and in the surface membrane of adjacent glial and-feet; in accordance with the original observation of Torack and Barrnett (1964), applying Wachstein-Meisel's method (1957) in their work. The different result of Rechard and Kokko (1967) may probably be explained so that these authors wanted to demonstrate first of all the ATP-ase activity of mitochondria sensitive to fixatives. Therefore they have carried out a formaldehyde perfusion lasting but a very short time (10 minutes), having visualized even the weak ATP-ase activity of the endo-

thelial cytoplasm. This result calls our attention to the fact that a weak ATP-ase activity can be found in the pinocytotic vesicles of vessels of the central nervous system, too; its function, however, is destroyed by being completely fixed as a consequence of striving after a perfect basic structure. Thus the apparently contradictory result does not change the basic observation described by Torack and Barrnett (1964).

In our earlier investigations about ChE activity of the brain capillaries we have found that the BuChE activity can be demonstrated only in capillaries of the brain fields defended by the blood-brain barrier (Joó and Csillik, 1966). In the course of our ontogenetical investigations, we have found a correlation between the development of the BuChE activity of brain vessels and the time of the blood-brain barrier complexed (Joó, Várkonyi and Csillik, 1967). As to the localization of the fine structure of ChE activity, we have not got an identical result, as using the "direct colouring" method, resp. that of Pb-thiocholine. In case of using the "direct colouring method" founded upon the reduction of ferricyanide, with acetylthiocholineiodide substrate on the surface of the endothelial cell, as well in cytoplasm and the basement membrane, alike, we find some precipitate that refers to the final product of reaction. With a butyrylthiocholineiodide substrate, supporting the data of Shimizu and Ishii (1966), we have found enzyme activity only in the basement membrane of the elements building up the capillary wall. During our investigations performed earlier with Pb-thiocholine methods (Joó and Csillik, 1966; Kása and Csillik, 1966; Joó, 1967), and also we in this study, have observed BuChE activity in the cytoplasm of endothelial cells, as localized mainly in the pinocytotic vesicles, and acetylcholinesterase activity in the basement membrane. At demonstrating Che activity with two different methods, we have obtained two different results concerning the localization of enzyme. As to the real cause of that, we are uncertain at present. It can be imagined that the potassiumferricyanide applied by the "direct colouring" technique has a peculiar affinity to a component of basement membrane, but it is possible, too, that at applying the method of Pb-thiocholine, the differing picture is resulted by the subsequent ammoniumpolysulfide convertation. For recognizing the precise localization of ChE activity in the cerebral capillaries, further investigations are necessary during which we can research the fine structural localization of enzyme with other methods, too, described for demostrating ChE activity in an electron histochemical way. We want to note that Shute and Lewis (according to non-published observations) found BuChE activity in the endothelial cytoplasm and acetylcholinesterase activity in the basement membrane, with the electron histochemical method elaborated by them — and in harmony with our result.

It is clear from the results described above, in which components of capillaries the non-specific alcaline phosphatase, ATP-ase, BuChE and AChE activity were found. The enzymes investigated were localized in the cytoplasm of endothelial cells, in basement membrane and in the surface membrane of glial end-feet close to the capillary. As it is known, in studying the penetration barrier of particular functioning in structures of the central nervous system, some authors held different components

of capillaries responsible for the development of the characteristic permeability relations. On the basis of electron microscopic investigations, some of them (Donahue and Pappas, 1961; Muir and Peters, 1962) consider the close connection of the endothelial cells with the so-called "tight junctions" as a morphological cause of the hematoencephalic barrier. Others (Friedmann and Elkeles, 1954; Rodriguez, 1955) are seeing the cause of the material supply slowed down in the particular structure of the endothelial layer of brain capillaries. Niessing (1952) is holding responsible the basement membrane surrounding the capillary wall continously, for inhibiting the barrier function. On the other hand, De Robertis and Gerschenfeld (1961) as well Edström (1964) emhasize the importance of the astrocyte end-feet system, that is close fitted to the basement membrane, for the functioning of the blood-brain barrier.

On this basis, therefore, we can not determine, in which degree the different enzymes of the capillary wall are taking part in forming the penetration barrier. At studying the permeability relations of the hematoencephalic barrier we have got to a result by applying some particular enzyme inhibitors. Grieg and Holland (1949) observed an increase of permeability of hematoencephalic barrier after administering eserine, we (Joó and Várkonyi, paper in preparation) observed the increase of the permeability of the blood-brain barrier after administering specific ChE inhibitors (Mipafox and/ or BW284C51) in cause of using a sensitive method founded on the fluorescence of trypane blue. We have observed a similarly increased permeability, as well, in case of investigating the permeability conditions of the hematoencephalic barrier after the ATP-ase activity being inhibited (Várkonyi and Joó, 1968). It is therefore referred to by our investigations that the ChE and ATP-ase activity of the capillaries is — in some way — in a functional connection with the function of the permeability barrier that inhibits the macromolecular transport.

We want to notice that our result according to which the ATP-ase activity of cerebral capillaries is in connection with the barrier function, is demanding a revision of Barrnett's earlier establishement (1964) that had concluded from the localization of ATP-ase activity in the basement membrane to a faster material supply there. A connection existing between ATP-ase activity localized in the surface membrane of astrocyte end-feet round capillaries and in basement membrane and the function of blood-brain barrier is emphasized also by our latter investigations. A fine-structural alteration is namely to be observed after the ATP-ase activity being inhibited that is referring the a possibility that the activity of ATP-ase localized in basement membrane under physiological conditions may have a role in preserving the molecular organization of basement membrane (Joó, 1968).

As to the significance of enzymes, that can be demonstrated in brain capillaries with histochemical methods, in view of inducing the state of an increased permeability in case of the blood-brain being experimentally damaged, we have some further investigations in progress.

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Plate I A—B: Electron histochemical localization of the non-specitic alcaline phosphatase activity in brain capillaries of the rat. The final product of reaction (arrows) can be observed mainly in cytoplasm and basement membrane. Gömöri's method (x 35 000).
C: Electron histochemical localization of the non-specific alcaline phosphatase activity discovered with Gömöri's method modified by Fredricsson. The final product of reaction is localized in the cytoplasm of endothelial cells, first of all in pinocytotic vesicles. (x 35 000).

Plate II A—B: Electron histochemical localization of adenosintriphosphatase activity in brain capillaries, after applying the method described by Padycula and Herman. The final product of reaction (arrows) can be found in the basement membrane and in the surface membrane of glial cells. (A:x 22 000; B:30 000.)

Plate III Electron histochemical localization of butyrylcholinesterase activity in brain capillaries, after carrying out the "direct colouring" method. The final product of reaction (arrows) can be observed in the basement membrane. (x 22 000).

Plate IV A—C: Electron histochemical localization of acetylcholinesterase activity after applying the "direct colouring" method. The final product of reaction (arrows) can be found mainly in basement membrane but here and there also in the cytoplasm of endothelial cells, in pinocytotic vesicles (marked arrow; x 30 000).

Plate V A—D: Electron histochemical localization of butyrylcholinesterase activity, after applying the method of "Pb-thiocholine". The final product of reaction (arrows) is localized only in cytoplasm of endothelial cells, mainly in pinocytotic vesicles. (A and B:x 22 000; C:94 000; D:110 000). Figs. C and D were prepared by an electron microscope JEM 6 C, in the Electron Microscope Laboratory of Central Medical Research Institute, Budapest.

Plate VI A—C: Electron histochemical localization of acetylcholinesterase activity after applying the method of "Pb-thiocholine". The final product of reaction (arrows) can be found mainly in the basement membrane. Enzyme activity can sometimes be observed in the surface membrane of cytoplasm of endothelial cells, as well (marked arrow). A:x 20 000; B:x 25 000; C:x 45 000).

Abbreviations:

L:lumen of capillary
End:cytoplasm of an endothelial cell
BM:basement membrane
My:myelinated axon
sv:synaptic vesicle
M:mitochondrion
pv:pinocytotic vesicle
N:nucleus
Gl:glial elements
RBC:red blood cell

PLATE I

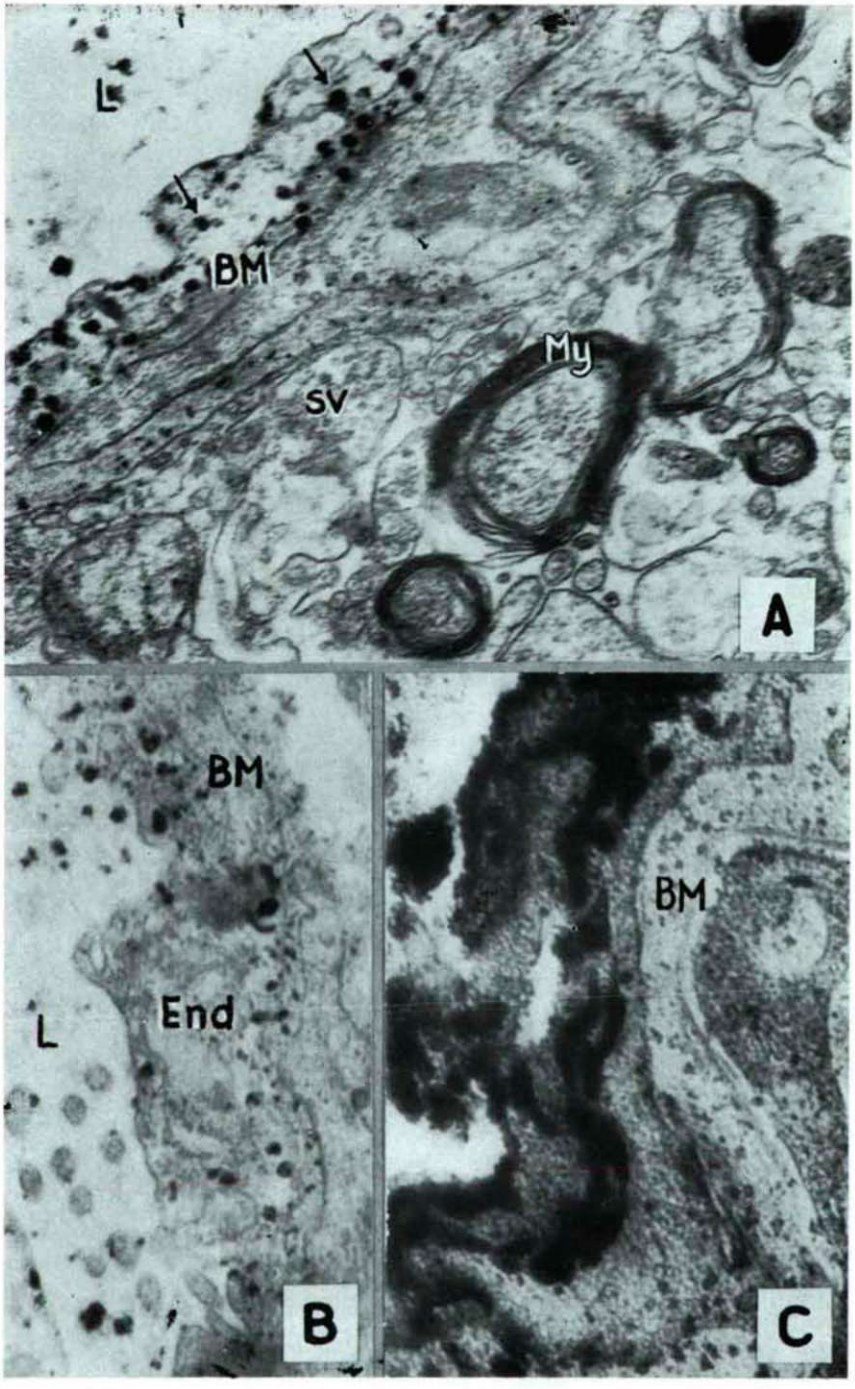

PLATE II

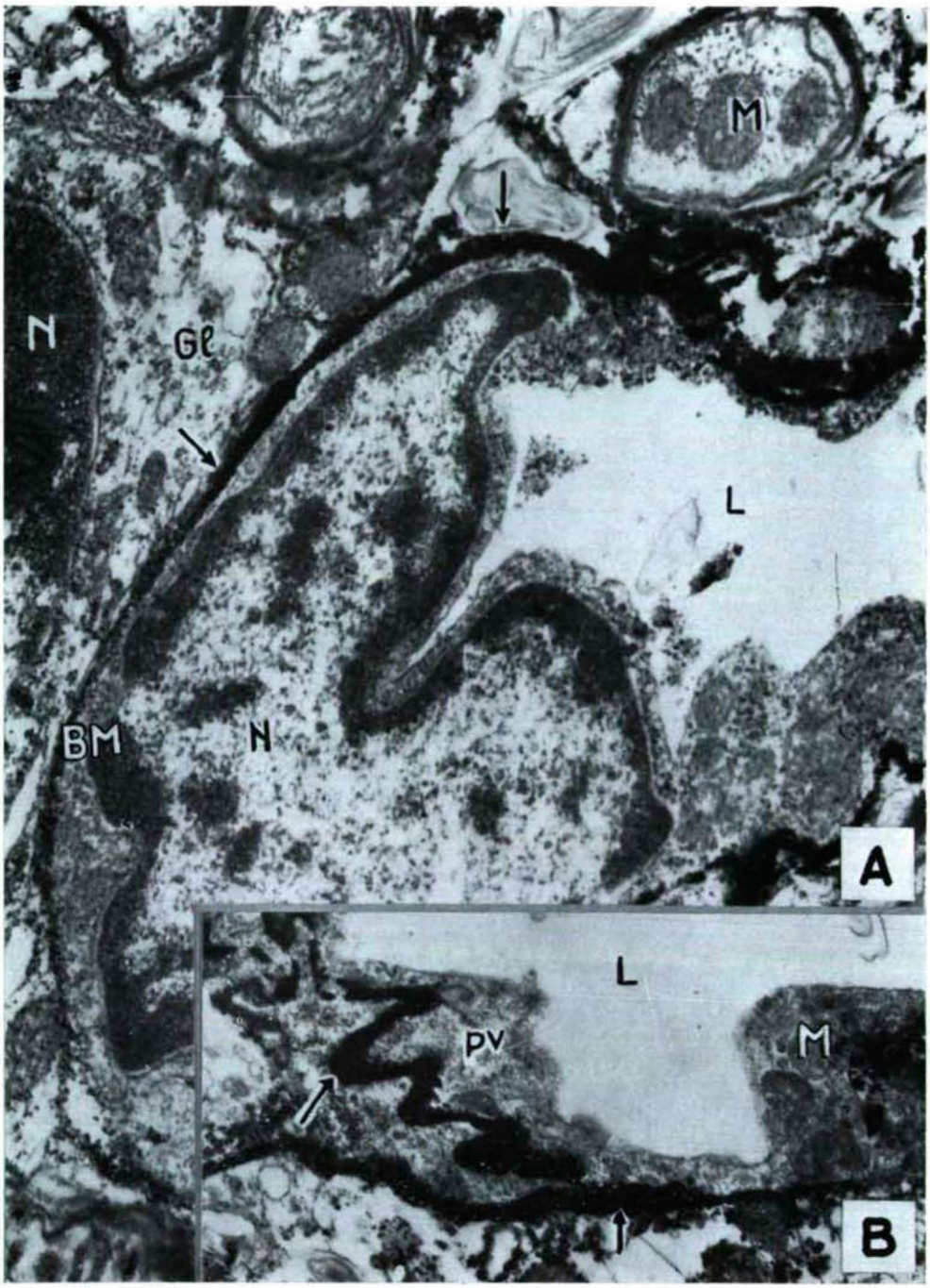

PLATE III

PLATE IV

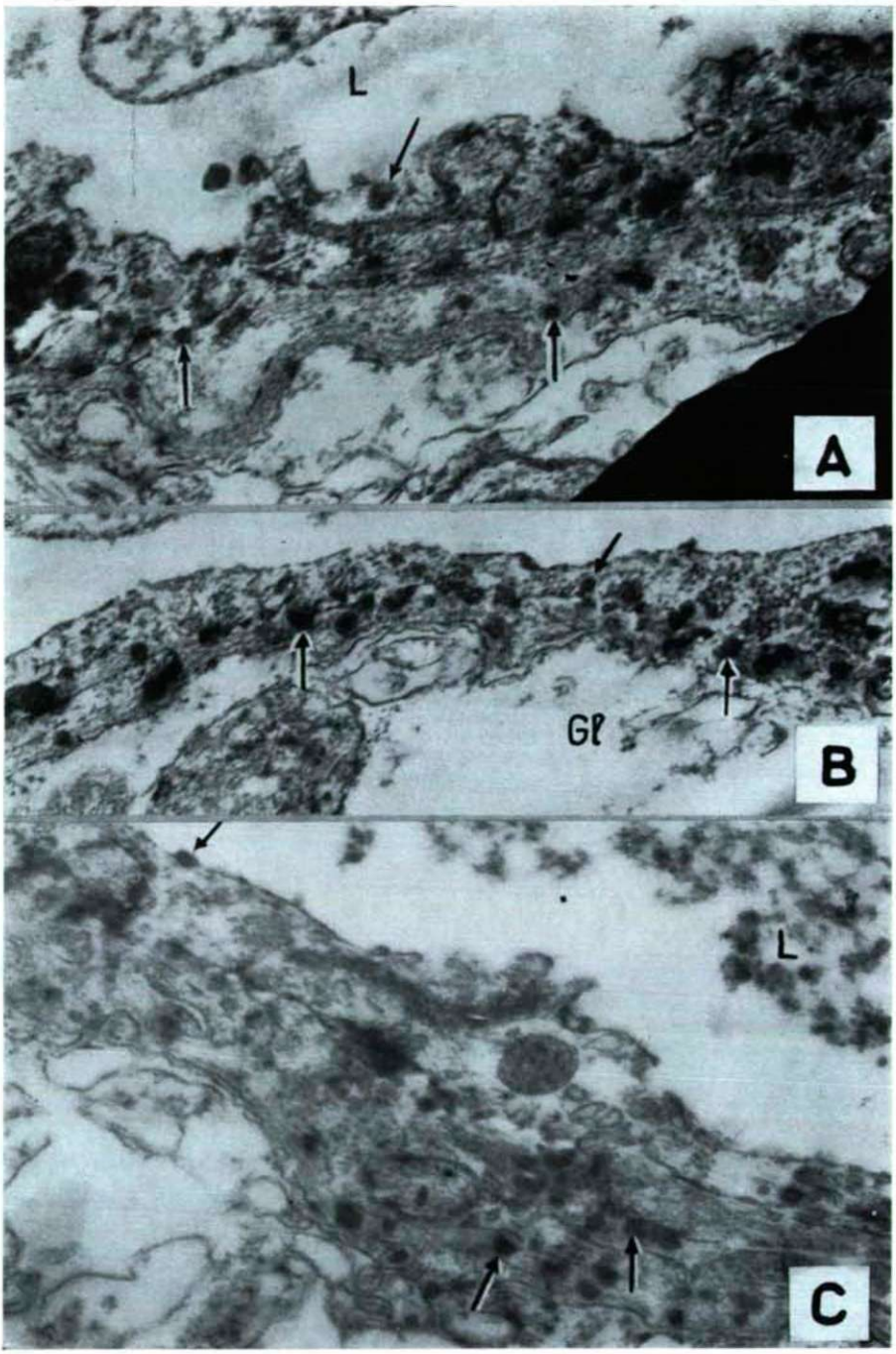

PLATE V

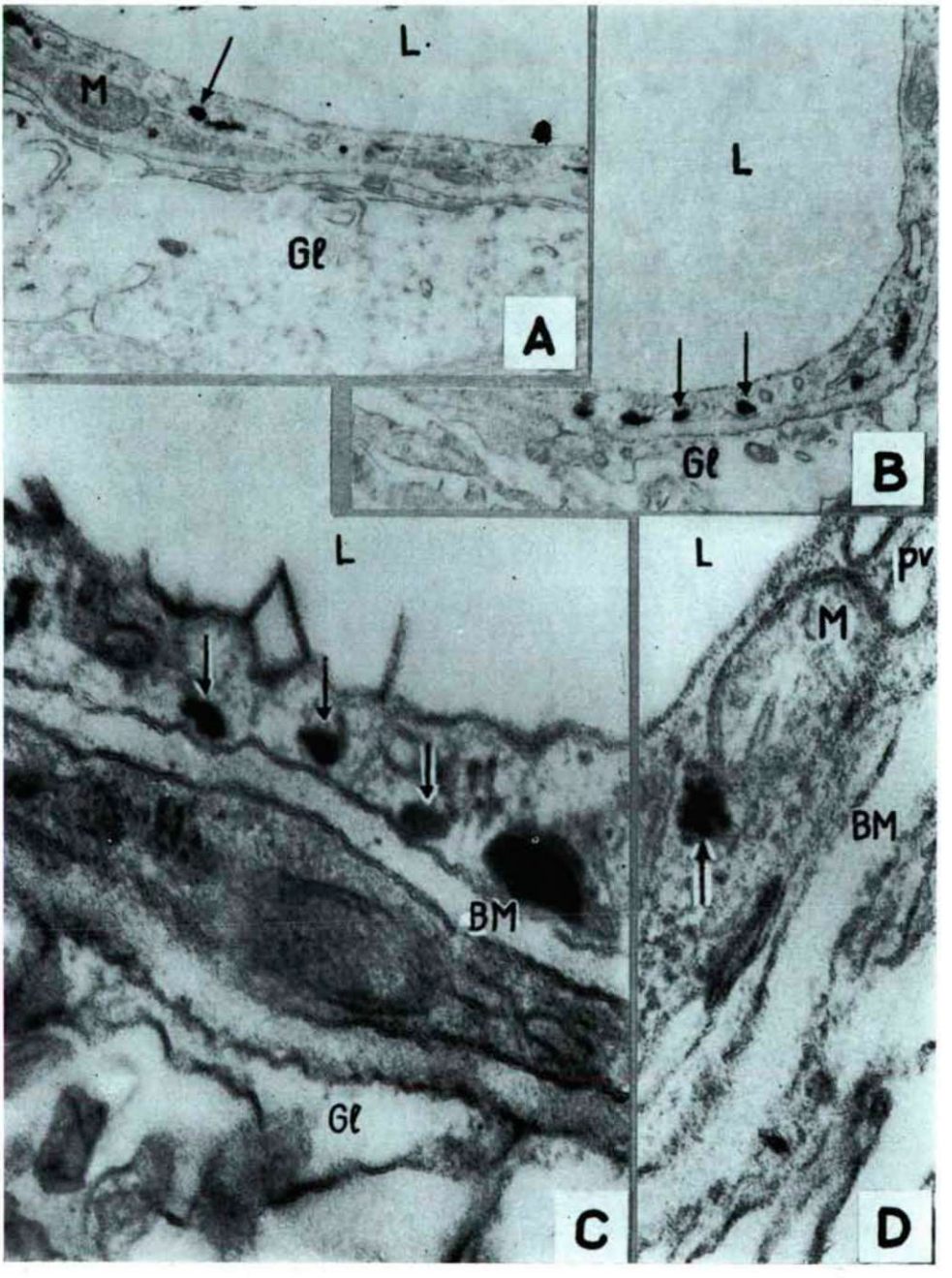

PLATE VI

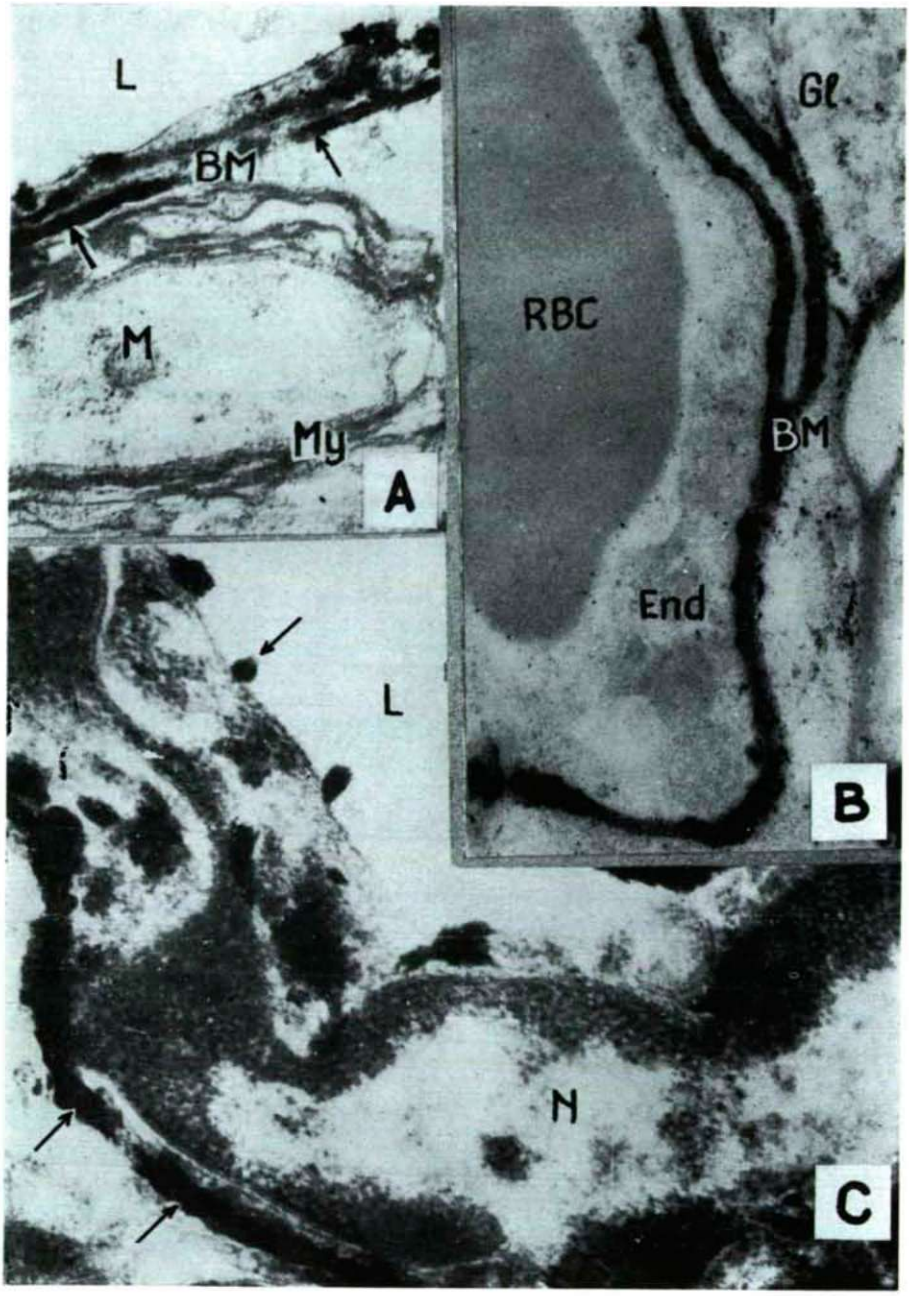

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PHOTO-INDUCED RESPIRATION OF THE SENSITIZED TETRAHYMENA PYRIFORMIS GL, INHIBITED WITH KCN

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Unicellular animals are multifunctional organisms. Their metabolism is, in a lot of connections, identical with that of *Metazoa*. That is verified by respiratory investigations performed on various *Protozoa* (Leichsenring, 1925; Wittner, 1957; Hall, 1938, 1941, etc.) and by the inhibition of respiration with its classic inhibitor, KCN (Pace, 1945; Reich, 1955; Sarojini and Nagabhushanam, 1966). For investigations like these a suitable culture of *Tetrahymena pyriformis* (Syn. *Tetrahymena geleii)* is very good (Pace and Lyman, 1947; McCashland and Pace, 1952; McCashland, 1956; Hunter and Hunter, 1957; G. van de Vijver, 1966).

The respiration can be influenced by different endogenous and exogenous factors; like temperature (Wittner, 1957; Pace and Kimura, 1944), pH (Sarojini and Nagabhushanam, 1966), the different effects of radiation and light (Giese, 1953). The light effects proved to stimulate the respiration temporarily. This phenomenon was obvious in the presence of adequate sensitors. The light-induced O_2-consumption of *Sacharomyces cereviseae* stained with Rose bengale was, e.g., much higher than otherwise and in the dark (Freaman and Giese, 1952). It proved instructive to be established how the respiration of *Tetrahymena pyriformis* was influenced by being light-sensitized, and the whole process by KCN.

Materials and Methods

The strain GL of *Tetrahymena pyriformis* was cultivated sterilely in the dark, in room temperature (22 $\pm$ 1.5°C), in a rat-liver extract. The stagnant cultures were inoculated in every third week. For the investigations cultures of 5—10 days were used. The substance was centrifuged twice and starved for 24 hours (McCashland and Pace, 1952) in a medium of defined ion-composition set to pH 6.9 with phosphate buffer (Biczók, 1961). The O_2-consumption, resp. the inhibition of the photoinduced respiration with KCN was measured at 25°C with the conventional Warburg technique (G. van de Vijver, 1966a,b). The animals were formerly sensitized with photodynamic substances of a concentration of to 50 000, namely with Rose bengale (RB) and methylene blue (MB).

5 ml substance (in the medium of the ioncompostion and pH mentioned) was poured into small vessels of 15 ml content, with 0.3—0.5 million animals. The concentration of the KCN inhibitor was $5 \cdot 10^{-4}$, resp. $5 \cdot 10^{-3}$M. The vessels were illuminated with two F-tubes built in the water-bath of Warburg's apparat, the luminous intensity of which was measured 1105 erg $cm^{-2}sec^{-1}$ (measured with an iron-constantan thermo-column of silica valve built into a vacuum, tested by measuring the radiated energy emitted by a 100 Watt wolfram electric bulb in a

1 m long black paper tube of 10 cm diameter (I. Horváth, Botanical Institute, A. J. University, Szeged, and J. Zimonyi, Biochem. Inst., Budapest). The shake-speed of vessels was 130 oscillation a minute. As a gas phase of measurement, atmospheric air was used. The exact number of animals after being measured and fixed in alcoholic formalin (the animals depose well), was established with Bürker's chamber, resp. Petri dish, wellproved in practice and applicable fast, putting a paper of sq.mm division on its bottom The cell respiration was denoted in the O_2-consumption per hour of a million organisms given in μl.

Result and discussion

1. Oxygen uptake without sensitor

The O_2-consumptions of the *Tetrahymena pyriformis* starved and non-starved, illuminated and breathing without light effect, have been compared on the basis of 142 measurements. The centrifuged animals cultivated in liver-extract have consumed 101 μl/mil. (h. oxygen) average of the quantities measured in the light and in the dark, in the anorganic medium. The average of the one hour oxygen consumption of the starved animals, measured in the light and in the dark, is 84 μl, that means 17 p.c. decrease opposite to the O_2-consumption of those well-fed.

2. Oxygen uptake in the presence of a sensitor

It is ascertained by the analyses of our direct measurements and photos that the speed of the movement of *Tetrahymena pyriformis* unstained and, all the more, of that sensitized with RB and MB, has considerably increased and then decreased as a result of light, depending on wave-length and intensity, and finally the animals have ceased moving; those of them that were sensitized were rounded off and often cytolysed. Temporarily also the number of frequency of the contractile vacuole increased (Biczók, 1966). In consideration of the photo-oxidation connected with these phenomena, it was to be expected that the oxygen uptake of sensitized animals as compared with those unstained increases. This supposition has been supported by our measurments (Table).

The numerical data have called the attention to some remarkable phenomena:

a) The respiration of the *Tetrahymena* unstained was increased by light, if only in a lower degree. That is obviously connected with the presence of the "photoreceptoric molecules" that may be responsible (as absorbing light and having a capacity of being induced) indirectly for the increase of the speed of motion following the photo-reception.

b) The MB sensitor that, in case of the *Tetrahymena pyriformis* suspended in tap-water, has considerably increased the O_2-uptake (49 p.c. — van de Vijver, 1966a), at our in vivo investigations has though resulted but in a stimulation of minor degree (in the dark 12 p.c., as influenced by light 20 p.c.), yet this stimulation has surpassed 4 p.c. that of RB known as of a stronger photodynamic effect.

Table 1. Inhibition of the respiration of Tetrahymena pyriformis, strain GL, unstained and sensitized, with KCN

	O_2-uptake as a result of light						O_2-uptake in the dark					
	Without inhib.	Stand. dev.	KCN 5.10^{-4}	Stand. dev.	KCN 5.10^{-3}	Stand. dev.	Without inhib.	Stand. dev.	KCN 5.10^{-4}	Stand. dev.	KCN 5.10^{-3}	Stand. dev.
Degree of inhibition			22 p.c.		28 p.c.				20 p.c.		24 p.c.	
Rose bengale (RB)	97 ul	± 6.8	76 ul	± 3.8	70 ul	± 2.1	87 ul	±6.6	70 ul	±3.1	66 ul	± 2.8
Degree of inhibition			17 p.c.		46 p.c.				15 p.c.		57 p.c.	
Methylene blue (MB)	101 ul	±10	84 ul	±14.7	55 ul	± 7.2	92 ul	±6.7	78 ul	±14	40 ul	±16.1
Degree of inhibition			10 p.c.		45 p.c.				16 p.c.		43 p.c.	
Unstained	87 ul	± 8.3	78 ul	± 8.8	48 ul	±10.4	81 ul	±9.1	68 ul	±6.4	46 ul	± 8.7

The respiration of Tetrahymena unstained for light has increased 7 p.c., of those stained with RB 16 p.c., of those stained with MB 20 p.c. (Values compared with those of dark unstained ones).

In the dark, RB has increased respiration 7 p.c., MB 12 p.c.

The respiration of those stained with RB has increased by light 11 p.c., of those stained with MB 9.8 p.c.

The intensity of respiration has considerably been influenced by the inhibitor KCN. On the basis of the Table it can be established that, depending upon the concentration of this compound, it inhibits not only the respiration of unstained animal but also that of the sensitized ones, in the light and dark equally. The inhibiting effect of KCN was not defended by the sensitors; the oxygen uptake has altered in about such a degree as it could be expected on the basis of the photodynamic character of stain.

Our results are in close connection with the statement that *Tetrahymena pyriformis* is cyanidesensitive (Hall, 1941) and that the degree of the respirative inhibition is expressed by the KCN-concentration (McCashland, 1952; 1956). The $5 \cdot 10^{-3}$M concentration of KCN, applied by us, has resulted in a significant inhibition; at the $5 \cdot 10^{-4}$M concentration there occurred here and there a stimulated respiration, as well.

KCN has inhibited not only the animals without sensitor but also the respiration of those stained with RB and MB, stimulated to the light more, to the dark less. The inhibition seemed to support the data published by Baker and Baumberger in 1941 (in Lwoff, 1951) concerning the role of cytochromes, of cytochrome-oxidase in the respiration of *Protozoa*. The resepiration is actually complicated. The investigations of van de Vijver (1966b) raised namely doubts as to the existence of cytochrome-c oxidase at *Tetrahymena pyriformis*. As the inhibition of KCN was not defended by MB the oxygen uptake has not increased at the animals inhibited as a result of MB, it seems reasonable to suppose the absence of cytochrome-c, resp. of cytochrome-c-oxidase.

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ZOOPLANKTONUNTERSUCHUNGEN IM ÖSTLICHEN-HAUPTKANAL

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Der Östliche—Hauptkanal ist ein die Theiss und Berettyó zusammenbindender 100 km langer künstlicher Kanal. Er entspringt von der Theiss, über der Staustufe zu Tiszalök (beim 525. Fluss km), in dem von dem Flusskrafwerk aufgestauten Teil; bei Bakonszeg mündet er in den Káló-Kanal, der sich nach einiger Kilometern in Berettyó giesst. Es kann aus dem Östlichen-Hauptkanal ein Gebiet von ung. 150 000 Katastraljoch bewässert werden, und zwar teils auf Graviatationsweg; er ist deshalb für die Volkswirtschaft eine sehr bedeutende Anlage.

Im Kanal hängt die Wassergeschwindigkeit und Menge davon ab, wieviel Wasser durch die von dem Ursprung aus der Theiss 5 km weit befindliche Schleuse zu Tiszavasvár überlassen, bzw. wieviel Wasser aus dem Kanal für Bewässerung herausgenommen wurde. Die durch die Schleuse überlassene Wassermenge verändert sich zwischen 0—50 m^3/sec. In der Wässerungsperiode ist die Wassergeschwindigkeit im Östlichen-Hauptkanal viel grösser als in der Theiss über der Staustufe zu Tiszalök. In dieser Zeit enthält das Wasser des Östlichen-Hauptkanals viel mehr Schwemmstoffe, die ganz trüb sind, mit einer Transparenz von nurmehr einigen cm. In Anderen Perioden steht sein Wasser beinahe völlig still, es ist klar, enthält nur wenige Schwemmstoffes, seine Transparenz erreicht selbst die 70—80 cm. In dieser Periode kann eine "Wasserblüte" beobachtet werden; die Oberfläche des Wassers wird von einer dicken Neustonhaut bedeckt.

Die systematische Untersuchung der Phyto- und Zooplanktons des Östlichen-Hauptkanals ist in 1963 begonnen worden, als wir die erste 40 km Kanalstrecke eingehend untersucht hatten. Das Phytoplankton wurde von Uherkovich (1964, 1966), das Zooplankton von Gál (1964) bearbeitet. Ich habe in 1964 in vier Punkten des Östlichen-Hauptkanals [Theiss (0. km), Schleuse zu Tiszavasvár (5. km), Hajdúszoboszló (70. km) und Berettyóújfalu (95. km)], in drei Zeitpunkten Sammlungen durchgeführt (Mai, Juli, November) um festzustellen, wie sich das Zooplankton des aus der Theiss hineingeflossenen Wassers im Östlichen-Hauptkanal qualitativ und quantitativ verändert (Abb. 1).

Untersuchungsmethoden

Die Sammlungen wurden mit einem Planktonnetz Nr. 25 ausgeführt. Ich habe bei jeder Gelegenheit 100 Liter Wasser durch das Planktonnetz gefiltert. Ich habe immer möglichst in der Mitte des Kanals, aus der 20—25 cm Oberwasserschicht gesammelt. Der gesam-

Abb. 1. Schematische Landkarte des Östlichen-Hauptkanals, mit der Bezeichung der Sammelstellen.

melte Stoff wurde an Ort und Stelle in Formalin fixiert. Während der Bearbeitung habe ich den gesammelten Stoff in einen 10 ml Meßzylinder gegossen und auf 10 ml ergänzt. Davon habe ich im allgemeinen 2 ml (das 20 Liter Wasser entspricht) völlig bearbeitet, und gezählt, wieviel Einzelorganismen von den einzelnen Arten zu finden sind. In einigen Fällen — hauptsächlich bei der Novembersammlung — hatte ich 5 ml zu bearbeiten, denn die Anzahl der Einzelorganismen war so klein. Ich habe bei jeder Gelegenheit nur soviel zu bearbeitenden Stoff auf den Objektträger gelegt, der von Deckglas völlig bedeckt wurde. Die Zählung habe ich mit Hilfe des Quertisches des Mikroskops ausgeführt und den vom Deckglas bedeckten Teil in Reichen überblickt. Die erhaltenen Ergebnisse wurden auf 100 Liter umgerechnet. Bei Untersuchung des Zooplanktons — besonders im Fall des Flußwassers, wo im allgemeinen

viel wenigere Tiere leben, als im Stillwasser — ist es Zweckmäßiger die Menge der in 100 Liter Wasser lebenden Tiere anzugeben, denn wir bekommen so ein viel genaueres Bild. Ich habe auch das prozentuale Vorkommen der einzelnen Arten ausgerechnet (die Gesamtanzahl der auf der Sammelstelle gefundenen Einzelorganismen wurde für 100 Prozent angenommen, und die Einzelzahlen der einzelnen Arten wurden dazu ins Verhältnis gestellt).

Besprechnung der einzelnen Sammlungen

Die genauen Ergebnisse der einzelnen Sammlungen sind in Tabelle 1 enthalten worden. Auf Abbildung 2 werden die erhaltenen Ergebnisse graphisch dargestellt.

I. 1. 14 Mai 1964: Die Theiß Ursprung des Östlichen-Hauptkanals. (Fkm 0). Wassertemperatur: 16° C, pH 6,8.
2. 14 Mai 1964: Der Östliche-Hauptkanaul bei der Schleuse zu Tiszavasvár. (Fkm. 5). Wassertemperatur: 16,3° C, pH 6,8.
3. 15 Mai 1964: Der Östliche-Hauptkanal zu Hajdúszoboszló. Wassertemperatur: 17,4° C, pH 6,8.
4. 16 Mai 1964: Der Östliche-Hauptkanal zu Berettyóújfalu. Es gibt im Wasser mehrere Schwemmstoffe als in den vorigen Stellen. Wassertemperatur: 17,7° C, pH 6,7.

Protozoa (haputsächlich *Testacea*-Arten): Ihre Gesamteinzelanzahl nimmt allmählich zu als wir in den Kanal nach innen vorwärstgehen, sie nimmt aber vor dem Ende des Kanals — zu Berettyóújfalu — stark ab. Dort habe ich nurmehr zwei Arten *(Centropyxis constricta* und *Cyphoderia margaritacea)* mit kleiner Einzelorganismenanzahl gefunden; sie bilden nur 0,14 % des Gesamtplanktons. Die vorgefundenen Arten sind in der Theiss allgemein verbreitet.

Rotatoria: Sie bilden die Hauptmenge des Zooplanktons, sowohl in der Hinsicht der Anzahl der Arten wie in der der Einzelorganismen. Die Anzahl der Einzelorganismen nimmt im Östlichen-Hauptkanal stark zu und erreicht das Maximum bei Berettyóújfalu. Zunächst einmal ist die *Enteroplea lacustris* die dominante Art: auf all den vier Sammelstellen bildet dies ein grosses Prozent des Zooplanktons. Die Anzahl ihrer Einzelorganismen nimmt im Östlichen-Hauptkanal sehr stark zu, bei Berettyóújfalu können in 100 Litern mehr als 25 000 Einzelorganismen gefunden werden, was 94,5 % des Gesamtplanktons ausmacht. Mit Ausnahme der Sammelstelle zu Hajdúszoboszló, es leben in den anderen drei Stellen im grossen und ganzen dieselben Arten. In der Stelle bei Hajdúszoboszló erscheinen hingegen völlig andere Arten, nur *Enteroplea lacustris* und *Trichotria quadrangularis* sind gemeinsame Arten. Die bei Hajdúszoboszló erschienene *Euchlanis dilatata* kann auch bei Berettyóújfalu aufgefunden werden.

Crustacea: Die Gesamtanzahl ihrer Einzelorganismen ist in der Theiss und beim Km. 5. im Östlichen-Hauptkanal ungefähr identisch. Bei Hajdúszoboszló springt die Gesamtanzahl der Einzelorganismen hoch empor, hauptsächlich wegen der Zunahme der Anzahl der Naupliuslarven, sowie wegen der Massenerscheinung des *Chydorus sphae-*

ricus. Bei Berettyóújfalu nimmt die Anzal der Einzelorganismen ab, sie ist aber noch immer 12-mal grösser als die Gesamtanzahl der Einzelorganismen in der Theiss.

Von den anderen Arten konnten in den ersten beiden Sammelstellen die Nematoden, in den anderen beiden Sammelstellen die Chironomiden und die Mückenlarven in kleiner Anzahl der Einzelnorganismen gefunden werden.

II. 1. 24. Juli 1964: Die Theiss beim Ursprung des Kanals. Wassertemperatur: 25,5° C, pH 6,8.
2. 20. Juli 1964: Bei der Schleuse zu Tiszavasvár. Wassertemperatur: 25,5° C. pH 6,8.
3. 21. Juli 1964: Bei Hajdúszoboszló. Wassertemperatur 26,3° C, pH 6,8.
4. 22. Juli 1964: Bei Berettyóújfalu. Wassertemperatur 26,5° C, pH 6,8.

Protozoa: Bei der Theiss und bei Berettyóújfalu habe ich keine *Protozoa* gefunden. Auch in beiden dazwischenbefindlichen Stellen kamen nur einige Exemplare etlicher Arten hervor, die nur ein sehr kleines Prozent des Gesamtzooplanktons bilden. Die bei Hajdúszoboszló zum Vorschein gekommene *Hyalosphaenia papilio* habe ich im Wassersystem der Theiss bis jetzt nur in Maros den 18. Dezember 1959 gefunden.

Rotatoria: Im Plankton dominieren auch hier die Rotatorien mit einer grossen Anzahl der Einzelorganismen. Beim Km. 5. nimmt die Geseamtanzahl der Einzelorganismen in grossem Masse ab, bei Hajdúszoboszló nimmt sie stark zu, übersteigt stark selbst die in der Theiss befindliche Gesamtzahl der Einzelorganismen. Bei Berettyóújfalu nimmt die Gesamtzahl der Einzelorganismen ein wenig noch weiter zu. Beim Km. 5. führt die sehr bedeutende Abnahme die Abnahme der Einzelorganismenanzahl des in der Theiss mit einer sehr hohen Einzelorganismenzahl dominierenden *Brachionus calyciflorus* fma. *amphiceros* herbei, die in der Theiss 8340 ind/100 Liter und 73,8 % des Gesamtplanktons bilden, während sie im Östlichen-Hauptkanal, beim Km. 5. 4 840 ind/100 L. und nur 50 % des Gesamtzooplanktons ausmachen. Bei Hajdúszoboszló und Berettyóújfalu nimmt ihre Anzahl weiter ab, sie wird pro 100 L. ungefähr 450 und sie macht ungefähr nur 3 % des Gesamtzooplanktons aus.

Crustacea: In der Theiss leben sie mit wenigen Einzelorganismen, hauptsächlich als Naupliuslarven. Im Östlichen-Hauptkanal nimmt ihre Anzahl mehr und mehr zu. Beim Km. 5. erscheint die *Bosmina longirostris*, die danach in allen Sammelstellen gefunden werden kann.

Von der Schleuse zu Tiszavasvári bis zum Ende können im Plankton die Chironomiden und Mückenlarven in kleiner Einzelorganismenzahl gefunden werden.

III. 1. 13. November 1964: Die Theiss beim Ursprung des Kanals. Das Wasser ist sehr rein, enthält nur wenig Schlemstoffe. Wassertemperatur: 6,7° C, pH 6,4.
2. 13. November 1964: Bei der Schleuse zu Tiszavasvár. Wassertemperatur: 6,6° C, pH 6,5.
3. 14. November 1964: Bei Hajdúszoboszló. Wassertemperatur: 8,5° C, pH 6,7.
4. 14. November 1964: Bei Beretyóújfalu. Viel mehr Schwemmstoffe als in den Vorigen. Wassertemperatur: 8,7° C, pH 6,8.

Vergleichen mit den beiden vorigen Sammlungen, leben im Plankton nur sehr wenige Tiere.

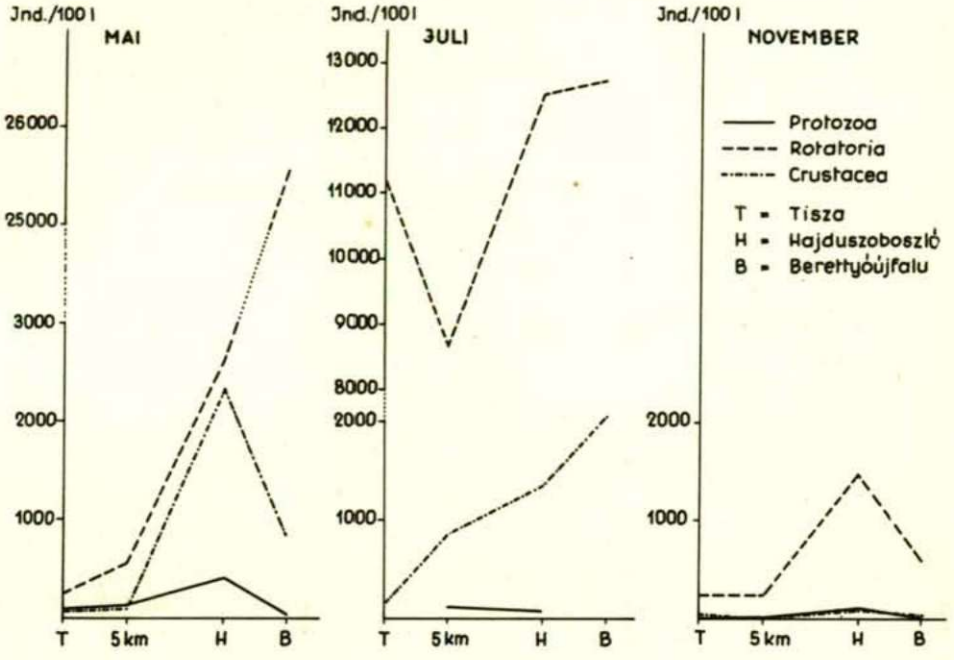

Abb. 2. Die quantitative Zusammenzetzung des Zooplanktons des Östlichen-Hauptkanals in den Monaten Mai, Juli und November.

Protozoa: Sie kamen in einer kleinen Arten- und Einzelorganismenanzahl vor, nur bei Hajdúszoboszló habe ich sie in einer grösseren Menge. Von den in der Theiss befindlichen beiden Arten *(Difflugia gramen* und *Arcella rotunda* v. *aplanata)* habe ich im Östlichen-Hauptkanal bei Hajdúszoboszló nur *Difflugia gramen* gefunden. Die im Östlichen-Hauptkanal gefundenen anderen Arten kamen dann von der Theiss nicht hervor. 2 Arten kamen im Östlichen-Hauptkanal überall zum Vorschein: *Codonella cratera (Ciliata)* und *Cyphoderia margaritacea (Testacea).*

Rotatoria: Sie bilden ungefähr 90 % des Planktons. In allen Sammelstellen kommen im grossen dieselben Arten vor. Die Gesamtanzahlen der Einzelorganismen stimmen in der Theiss und beim Km. 5. überein, nehmen bei Hajdúszoboszló stark zu, dann bei Berettyóújfalu wieder ab.

Crustacea: In der Theiss und bei Berettyóújfalu lebte die *Bosmina longirostris* mit wenigen Einzelorganismen. Ausserdem fand ich nur Naupliuslarven auf allen Sammelstellen in kleiner Einzelorganismenzahl.

Von den anderen Arten haben in der Theiss und im Östlichen-Hauptkanal beim Km. 5. Nematoden in kleiner Einzelorganismenanzahl gelebt.

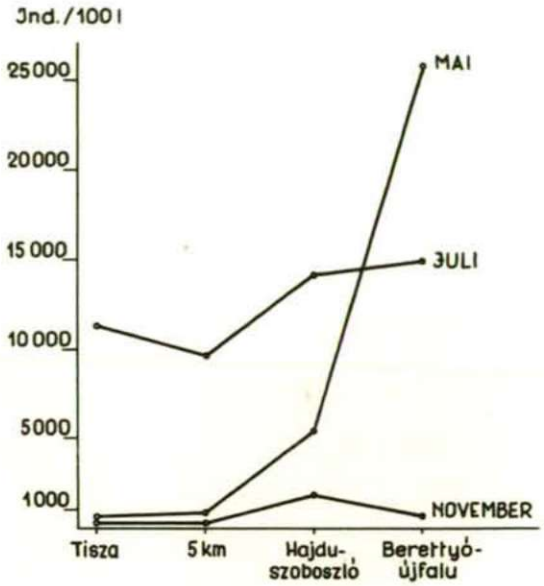

Abb. 3. Die Veränderung der Gesamtanzahl der Zooplanktoneinzelorganismen in den Sammelzeitpunkten im Östlichen-Hauptkanal.

Zusammenfassung

Auf Grund von in drei verschiedenen Perioden ausgeführten Sammlungen (Frühling, Sommer, Herbst) können über das Zooplankton des Östlichen-Hauptkanals die Folgenden festgestellt werden. (Abb. 3, Tabelle 1, 2, 3).

Die Protozoen kommen immer in kleiner Arten- und Einzelorganismenanzahl vor, beinflussen das Bild des Gesamtzooplanktons nicht wesentlich. Auch im Östlichen-Hauptkanal leben die in der Theiss allgemein verbreiteten Arten. Die am häufigsten vorkommenden Arten sind die *Centropyxis constricta* und die *Cyphoderia margaritacea*.

Mit der grössten Arten- und Einzelorganismenanzahl kommen die Rotatorien vor. In der Gesamtanzahl der Einzelorganismen dominieren sie immer im Plankton. Im Östlichen-Hauptkanal ist die Gesamtanzahl ihrer Einzelorganismen immer höher, als in der Theiss, ausgenommen die Julisammlung, als ihre Anzahl bei der Schleuse zu Tiszavasvár der starken Abnahme der Einzelorganismenanzahl des *Brachionus calyciflorus* f. *amphiceros* zufolge sehr abgenommen hat; aber sie hat auf den anderen zwei Sammelstellen die Gesamtanzahl der Einzelorganismen in der Theiss schon wieder überholt. Im Östlichen-Hauptkanal kommen die folgenden Arten ständig vor: *Filinia major, Keratella cochlearis, Keratella cochlearis* v. *tecta, Brachionus angularis, Enteroplea lacustris.*

Unter den allgemein Verbreiteten Arten vermag auch die *Polyarthra vulgaris* erwähnt zu werden, die aber bei Hajdúszoboszló konsequent fehlt.

Die niedrigeren Krebse können im Östlichen-Hauptkanal immer mit mehreren Einzelorganismen gefunden werden als in der Theiss.

Die Gesamtanzahlen der Einzelzooplanktonorganismen haben die grösste Abweichung in der Theiss und im Östlichen-Hauptkanal im Mai gezeigt. Während die Gesamtanzahl der Einzelorganismen in der Theiss und im Östlichen-Hauptkanal beim Km. 5. sehr niedrig war, hat sie am Ende des Kanals sehr stark zugenommen. Die in der Theiss und bei der Schleuse zu Tiszavasvár gefundene 459 ind./100 L., bzw. 800 ind./100 L. Einzelorganismenzahl hat bei Berettyóújfalu 25 000 ind./100 L. überholt.

In den Juli- und Novemberperioden sind die Theiss und der Östliche-Hauptkanal hinsichtlich der Gesamtanzahl der Einzelorganismen nicht wesentlich verschieden; die Gesamtanzahl der Einzelorganismen ist überall beinahe identisch: in Juli zwischen 10—15 000 ind./100 Liter, in November zwischen 250—1 670 ind./100 Liter wechselnd.

Die obigen Ergebnisse also zeigen, dass im Frühling die Geseamtanzahl der Zooplanktonorganismen in den Östlichen-Hauptkanal hineingehend stark zunimmt, im Sommer überall mittelmässig und in November sehr niedrig ist.

Taxonomischer Teil

In der Theiss und den Nebenflüssen habe ich die folgende Euglyphaart sehr oft gefunden:

Euglypha tiscia n.sp.

Gestalt und Struktur der Schale stimmen mit den der *Euglypha alveolata* überein. Der äussere Rand der um die Mundöffnung befindlichen Plättchen ist ausgezackt. Am Ende des Randes sind 4 kurze, nach innen gebogene *spinae* zu finden.

Die Länge der Schale ist 100—105 μ, ihre Breite: 50—54 μ. Sie ist eine in der Theiss nur zerstreut, mit wenigen Einzelorganismen vorkommende Art.

Ihre bisherigen Fundorte sind:

1. 14. November 1959: In der Theiss bei Tokaj ober- und unterhalb der Bodrogmündung.
2. 18. Dezember 1959: Bei Szeged in der Theiss unterhalb der Marosmündung.
3. 6. Oktober 1960: In der Kőrös bei Gyoma.
4. 2. Juni 1961: In der Theiss bei Tokaj unterhalb der Bodrogmündung.
5. 18. September 1961: Bei Szolnok in der Theiss oberhalb der Zagyvamündung.
6. 18. November 1961: In der Theiss bei Szolnok unterhalb dem Abwasserauslauf.
7. 27. Februar 1962: Oberhalb von Szolnok und unterhalb von Szolnok in der Theiss.
8. 24. Juli 1963: Im Östlichen-Hauptkanal bei Hajdúszoboszló und Berettyóújfalu.

Wie es sich aus den Obigen ergibt, kommt sie zunächst in den Wintermonaten — in einem kälteren Wasser — öfters vor, manchmal kommt sie aber auch im Sommer hervor.

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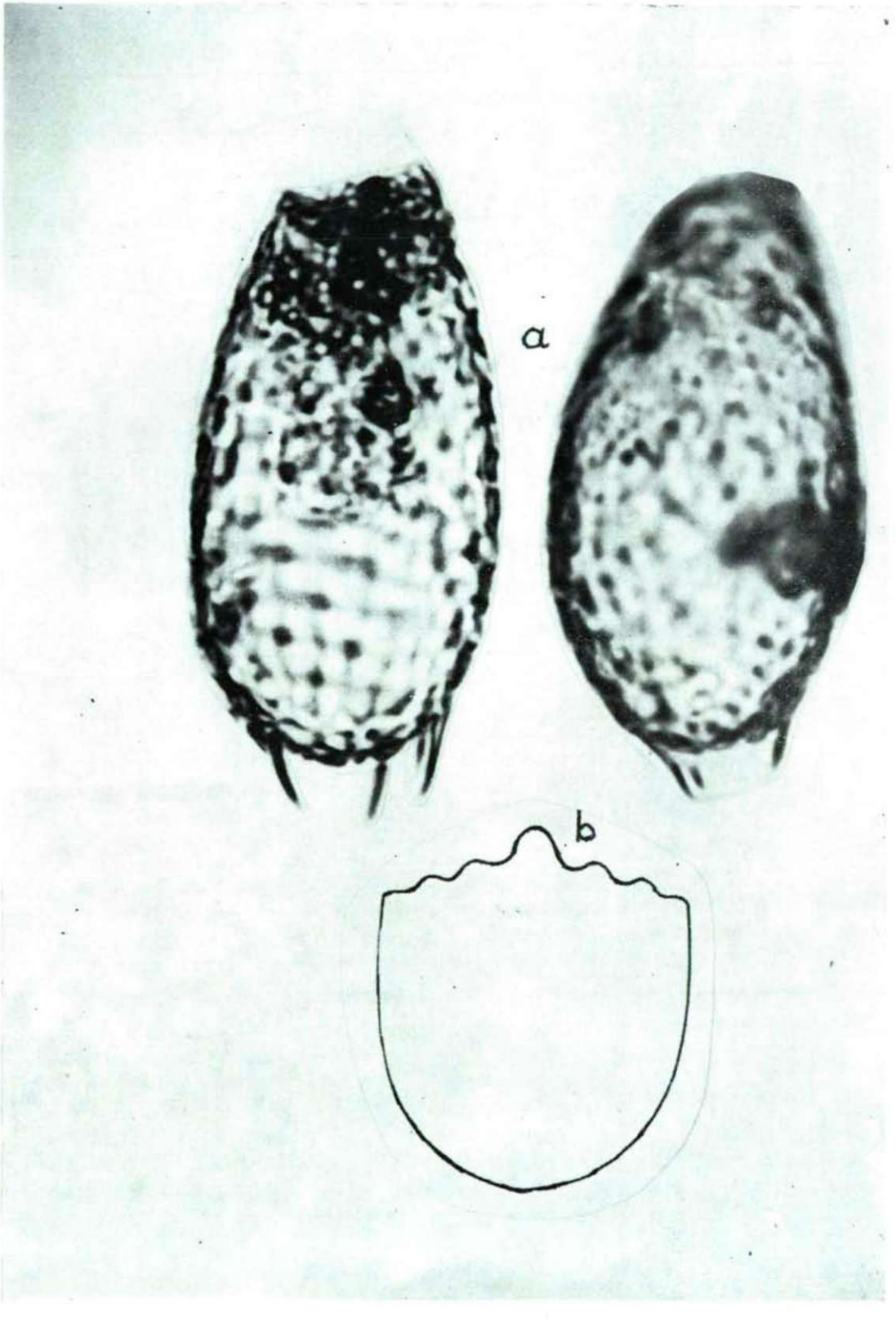
Tabellen 1. 2. 3. Die qualitativen, quantitativen und prozentualen Zusammensetzungen des Zooplanktons des Östlichen-Hauptkanals.

Tafel I.

a. *Euglypha tiscia* n.sp. (Mikrophoto)
b. *Euglypha tiscia,* Platte um die Mündung.

TAFEL I



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COMPARATIVE INVESTIGATIONS ON THE CILIARY GANGLION OF FRESH-WATER FISHES

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The occurance of ciliary ganglia in fishes is, according to the data of literature (Haller-Hallerstein, 1934; Hirt, 1935; Kolmer-Lauber, 1936; Krause, 1922), much debated. Some have not found it at all (Allborn, Cords, Ónodi), others publish it as a "real" ganglion (Tretjakoff, Herrick, Pankratz, Schneider), often in the form of a double ganglion (Schwalbe, Norris and Hughes). Some have observed it in the form of dissipated neurons, in the course of the oculomotor nerve or in the *ramus ophthalmicus* of trigeminal nerve. In case of our fresh-water fishes, where I have endeavoured to decide the question on the basis of comparative investigations, I found the following situation.

Anatomical situation of the ciliary ganglion

It is made difficult to recognize the ciliary ganglion of fishes by the fact that in these animals the cerebral nerve and the ganglia belonging to them, as well the upper part of the sympathetic nervous system and their connection with the organ of vision have a peculiar position. Therefore the sections, performed as in case of higher vertebrates, bringing the eyes to the surface together with the eye muscle cone from the very bottom of the orbital cavity and trying to find therein the ciliary ganglion, proved to be fully unsuccessful. The *ganglion ciliare* of fishes can be looked for only by dissecting thoroughly the anterior parts of the brain and maintaining the cerebral connections.

For finding it, we have to begin the dissection at the complex group of trigeminal and facial ganglia at the basal limit of the fore- and middle brains. In case of the carp we can observe particularly well two nerves that are leaving the upper ganglial group and running on the superficial surface of the eye muscle cone. The thinner is the superior ophthalmic branch of more cranial course and the thicker and more caudal the *ramus ophthalmicus profundus* (Fig. 1).

The *nervus oculomotorius* enters the orbital cavity through the opening lying proximal behind the *r. ophthalmicus* profundus. After a course of 1—2 mm it is divided and disappears in the eye muscle cone. If we examine carefully the place where the *n. oculomotorius* and the

r. ophthalmicus profundus are the next, we shall see macroscopically an obvious thickening and several very thin branches. In the thickening can be observed double ganglia with a stereo-microscope. The upper larger ganglion takes place in the course of the *r. ophthalmicus profundus*. The lower minor one is lying on the *n. oculomotorius* with its thicker end but it is attached inseparably to the upper ganglion with its connective tissue sheath and the nerve trunks running beside it. It is shown clearly by the histological examinations that there are two ganglia in question that are fully different from each other as to their structure and function. The upper one is the *ggl. opthalmicum profundum*, of cerebro-spinal character. Their myelinated fibres are running beside the smaller ganglion without establishing any contact with it (Fig. 1b). The smaller ganglion can be named — on the basis of its structure and nerve connections — correctly a ciliary ganglion. The 5—6 very thin nerve trunks that leave the ganglion make the ganglion star-like. These thin nerve-trunks, that can be considered as *nn. ciliares breves*, are getting to the *sclera* in the sheath of the *n. opticus* or in its neighbourhood. They are continuing their way to the ciliary muscle for the greatest part in the *sclera* and only for the smaller part in the *chorioidea*. From the ganglia of *n. trigeminus* several thin nerves are attaching the trunks entering the *sclera*. These are the long ciliary nerves *(nn. ciliares longi)* preserving their independence in the whole course.

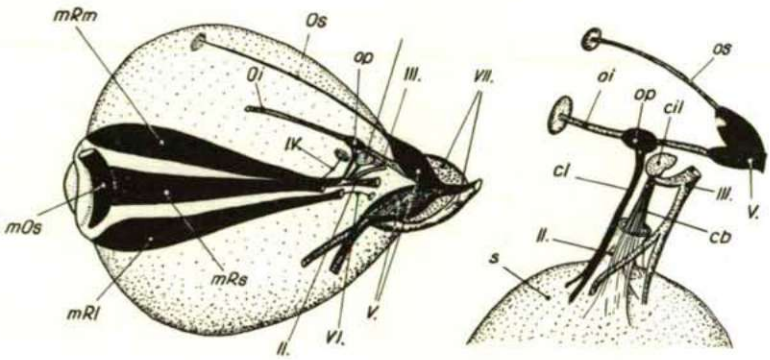

Fig. 1. *Cyprinus carpio:* The site of the ciliary ganglion and its relation to the cerebral nerve courses. II — VII. cerebral nerves and their ganglia, os — *ramus ophthalmicus superior*, oi — *ramus ophthalmicus inferior*, op — *ganglion opthalmicum profundum*, cil — *ganglion ciliare*, mR-MO — eye-muscles, cb — *nervi ciliares breves*, cl — *nervi ciliares longi*, sk — *sclera*.

At looking for the ciliary ganglion of the fishes and investigating their nerve-connections, we have to pay a special attention to the cranial part of the sympathetic nervous system. As it is obvious from the literary data, enumeratd above, some authors are considering the ganglion to belong to this system, resp. to obtain roots from here, too. The cranial sympathetic system of fishes is highly peculiar. The most

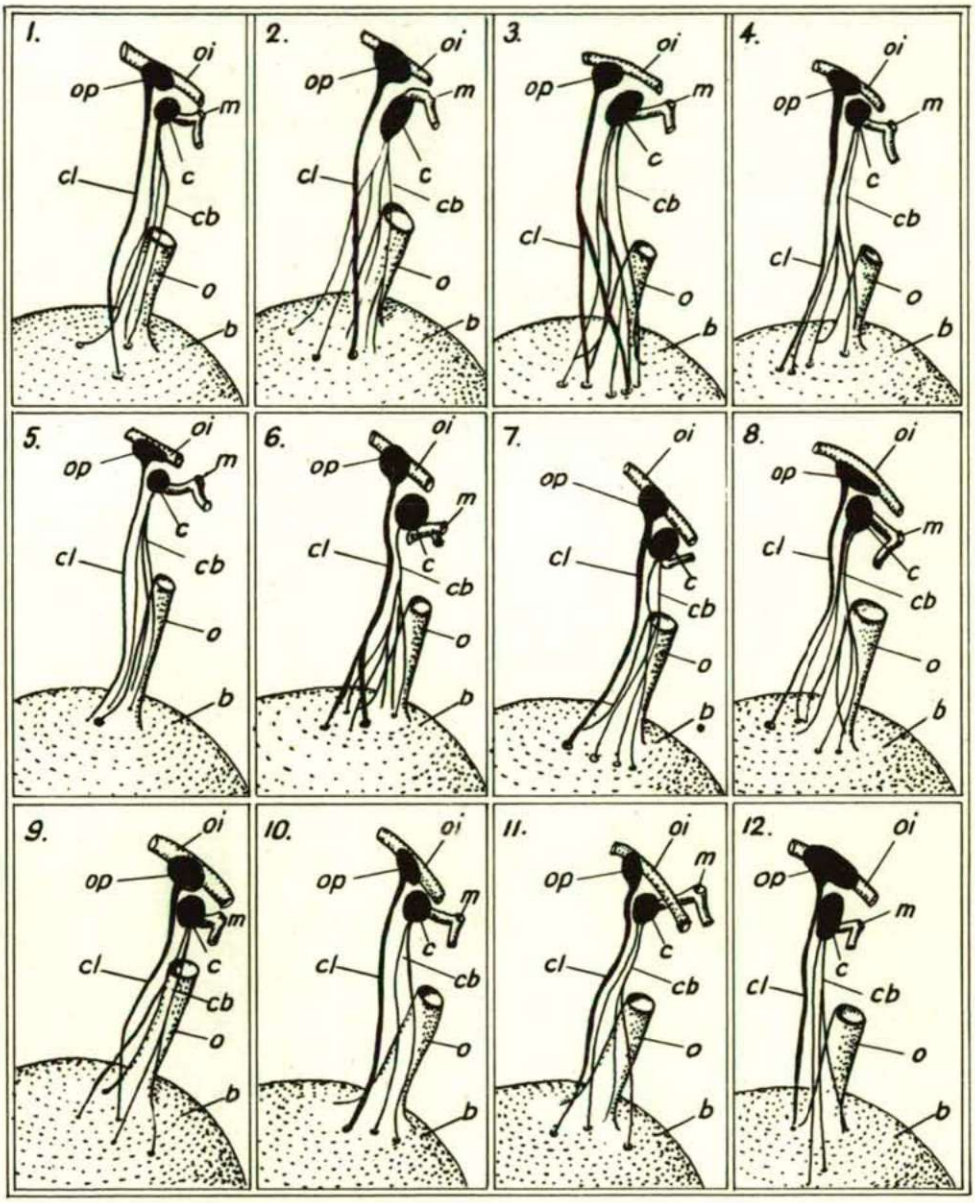

Plate I

The nerve connections of the ciliary ganglion of fishes: b — *bulbus*, c — *ganglion ciliare*, o — *nervus opticus*, m — *nervus oculomotorius*, cb — *nervus ciliaris longus*, oi — *ramus ophthalmicus inferior*, op — *ganglion ophthalmicum* profundum.

1. *Acipenser ruthenus*, 2. *Salmo trutta*, 3. *Esox lucius*, 4. *Carassius carassius*, 5. *Barbus barbus*, 6. *Rutilius rutilus*, 7. *Abramis brama*, 8. *Tinca tinca*, 9. *Cyprinus carpio*, 10. *Misgurnus fossilis*, 11. *Silurus glanis*, 12. *Perca fluviatilis*.

obvious and interesting difference from those of the higher vertebrates is that here the cephalic part of the sympathetic trunk is running on the base of the skull near to the cranial nerves highly possible the connection between the two systems.

In carp heads of large size, in which I have dissected out also the ciliary ganglion, I had the opportunity to investigate also the cranial sympathetic system. The cranial system can be observed beside the *arteria basialis* that is running on the brain-base and its connection with the ciliary ganglion was found as follows.

The uppermost three sympathetic ganglia are lying quite close to one another, they almost seem to be fused; the fourth one is located a little farther, and the fifth one much deeper on the base of the cranium, about so high that it corresponds to the height of the *ggl. cervicale supremum* of the reptiles and birds but higher than the uppermost cervical ganglion at the most part of mammala and at man. From the cranial sympathetic ganglia of fishes several nerves are running to sheaths of the adjacent cerebral nerves. It could be established with the microscopic investigations that not only nerve fibres but also sympathetic nerve cell groups can be found in the course of these nerves. From the uppermost cranial sympathetic ganglion, the most fine branches run in the wall of the ophthalmic artery. The plexus formed in the vessel-wall is very rich in fibres and nerve cells can be observed in it too. Some of the fine thin sympathetic branches are advancing towards the eye, others are running on the immediate surface of the optic nerves. In our opinion, some of these fibres can join the rich plexus system developing at the meeting point of the *nn. ciliares breves* et *longi;* it could, at any rate, not be established either macroscopically or on the basis of the microscopic structure, that the cranial sympathetic system has a direct connection with the ciliary ganglion.

Comparative anatomical data

We have dissected the ciliary ganglia of fish species of different ways of life and belonging to various families. According to the investigations, there is some difference in site and shape of ganglia, as well in the number and course of the postganglionic fibres (Plate I,1—12). The most obvious differences are, however, to be seen in the size of ganglia.

THE SITE OF GANGLION changes because of its relation to the *ggl. ophthalmicum profundum.* While in case of the carp *(Cyprinus carpio)* the two ganglia nearly fused, there are species where the two ganglia are farther from each other (Plate I,2,3,6,11,12). Owing to this change of site, mostly the shape of ganglion changes, as well.

THE SHAPE OF GANGLION is generally longish and oval (Plate I2,3,12) which reminds us generally of the ciliary ganglia of birds; sometimes, however, it is fully round (Plate I,5,8,10). Tri-, resp. quadrangular forms don't belong to the rarities, either (Plate I,1,4,7,9,11).

THE POSTGANGLIONIC NERVES are everywhere very thin and their number is very high. Immediately after leaving the ganglion the plexus formation of postganglionic fibres begins. From the postganglionic nerves there are running a great lot of fibres through the sheath of the optic nerve to the superficial connective tissue layer of the *sclera* and towards the *cornea*. Apart from entering by the sheath of the optic nerve, some nerves enter the eyeball through the *sclera* to the *chorioidea* as well. These branches are less numerous and somewhat thicker in size. In the number of the postganglionic nerves there are obvious differences. At the mudfish *(Misgurnus fossilis)* we see only two nerves, at the tench *(Tinca tinca)* three ones. At these species, the number of side branches is low, too (Plate I,4,11). At the others, the number of the postganglionic nerves is 6—12; because of the side branches, however, in the sheath of the optic nerve and at entering the *sclera*, as many as 14—22 can be counted.

Species	Ciliary ganglion			The greatest diameter of the eyeball	Number of relation	Order of sequence
	length	width	average			
Acipenser ruthenus	0.6	0.3	0.45	10	22	13
Salmo trutta	1.6	0.5	1.05	15	14.2	5
Esox lucius	1.6	0.8	1.2	14	11.6	1
Carassius carassius	0.9	0.5	0.7	13	18.5	10
Barbus barbus	0.8	0.7	0.75	12	16	6
Rutilus rutilus	0.8	0.8	0.8	11	13.7	4
Abramis brama	0.8	0.6	0.7	13	18.5	9
Tinca tinca	0.7	0.7	0.7	14	20	12
Cyprinus carpio	1.1	0.5	0.8	15	18.7	11
Misgurnus fossilis	0.5	0.5	0.5	8	16	7
Nemachilus barbatulus	0.6	0.5	0.55	9	16.3	8
Silurus glanis	0.3	0.3	0.3	7	23.3	14
Amiurus nebulosus	0.3	0.2	0.25	7	28	15
Perca fluviatilis	1.6	0.6	1.1	14	12.7	2
Lucioperca lucioperca	1.4	0.8	1.1	15	13.6	3

The nerve fibres coming from the trigeminal nerve are only attached to the postganglionic fibres of the ciliary ganglion. The trigeminal connection seems to derive mainly from the *gg. ophthalmicum profundum*. The number of the nerve branches leaving this ganglion and connected with the postganglionic plexuses of the ciliary ganglion is usually one, exceptionally two (Plate I,3,10,12). Their side branches appear only in the confused network of the plexus system and are running, as demonstrated, in the nerve trunks of *sclera*, in the same nerve-trunk as the postganglionic nerve fibres.

THE SIZE OF GANGLION is very heterogeneous. Among the differences shown in the ciliary ganglia of various fish species, the differences in size are the most remarkable. This phenomenon is getting the most obvious if we dissect and compare with each other the ciliary ganglia of same sized species (about 25 cm).

As a result of comparsion, we have found so that the sizes of eyes and ganglia are not always in the closest connection with each other, as it is demonstrable by the values contained in the Table below. The data of Table want to express relative sizes. The longitudinal and latitudinal averages of the ganglion are compared with the greatest diameter of the bulb.

Bringing the results obtained in connection with the way of life of the animal species, we are receiving considerable data. The order or sequence of the relative numbers is, namely, referring to the closest connection with the way of life. The smallest relative number, i.e., the comparatively largest ganglion can be found in the pike *(Esox lucius)*. The large and strongly differentiated external and internal eye muscles and skeleton muscles are in connection with the predatory way of life, the fast swimming. The next one is the size of ciliary ganglion of the perch *(Perca fluviatilis)* and the next the pike-perch *(Lucioperca lucioperca)* they are alert even in the mud, watching for the prey and falling quickly on it. The latter is almost identical with the minnow *(Rutilus rutilus)* of a well-known excellent eye-sight.

We have found so that at fish of twilight and night activity not only the eyes but also the ciliary ganglia are of great size, too. This is effecting first of all the trout *(Salmo)* that become alert at twilight and swim for getting food in the evening hours. Their alertness, dexterity and falling on the victim is characteristic of their getting the food. If in their youth they content themselves with getting insects, worms, snails and tadpoles, in their developed age they rival the outspokenly predacious fish in greed. From the species investigated, the ganglion of the barbel *(Barbus barbus)* of night life is the next one. The sizes of ganglia of the alert and agile meadow mudfish *(Misgurnus fossilis)* and those of the stone mudfish *(Nemachilus barbatulus)* swimming the whole night after its prey are of middle value. It is highly interesting that in the species belonging to the family *Cyprinidae* the ciliary ganglion is small. The most of them are actually unambitious animals.

On the basis of comparative investigations we have come to the conclusion that the ciliary ganglion of fishes are hardly changing with the age and body-size. As we dissected the ciliary ganglia out of large-sized carp heads, they were not larger, too, than those got from carps of smaller body-size. In view of size of ganglia, the sturgeons and siluruses are among the last ones. The strugeon *(Acipenser ruthenus)* is looking for its food on soft bottoms, half digged in, rather crawling than swimming. Siluruses *(Silurus glanis)* have a similar way of life. It can be established that where the other organs of sense are well developed, the eye and its nerve components are less developed. Thus, in case of the licking, touching strugeons and siluruses that taste the material of the muddy bottom with well-developed barbs and a trunk-

like mouth-piece and corresponding to these, supposedly with well-developed mechanical and chemical organs of sense, the size of eyes and that of the ciliary ganglia are particularly small. It is convincingly verified by the comparative data of the Table that the sizes of eyes and ciliary ganglia that belong to them are strongly influenced by the way and possibility of life and feeding.

Microscopic structure of the ciliary ganglion

According to the literary data the microscopic structure of the ciliary ganglia of fishes has not been investigated by anybody, as yet, and almost the same can be said also about the sympathetic and cerebrospinal ganglia. As the result of the anatomical investigation about the "existing" ciliary ganglion in our fresh-water fishes could only be decided with the help of the microscopic structure, we have made a careful examination concerning it. The literary data missing we have made a comparison for recognizing the structure of the ciliary ganglion and establishing its connections. For being able to compare correctly, we have examined also the structure of the cerebrospinal sensory ganglia and that of the sympathetic ganglia. As a result of the investigations we have established that the ciliary, cerebrospinal and sympathetic ganglia of the carp, the species of fishes investigated the most carefully, are structurally differring from one another.

CILIARY GANGLION. The cells of the ciliary ganglion of fish are unipolar. The cell process is thin, appearing but with difficulty even after being impregnated. The size of the cells is 10—15 μ, their staining being similar. The protoplasm of the cells is granular; the size of granules is as compared with those of higher vertebrates, much smaller. In the cell plasm the neurofibrils are very fine (Plate II, 1). The *nucleus* is comparatively large, its chromatin substance is rich. The *nucleolus* is separated from the nuclear substance only rarely. The cells are located densely near to one another; the fibre substance of the ganglia consists of thin fibres; a very thin myelin is only on the fibres of the nerve entering the ganglion. Round the cells granular glial cell nuclei take place. Their number is not high, round a cell 6—8. In the connective tissue around the ganglion and among the cells, near to the cellbody or on the cell-body itself there appear terminal heads, terminal clubs in high number (Plate III,1,2). These end-formations are considered to be preganglionic fibre terminations. Their size is 0.2—2 μ, mostly also the nerve fibre connection can be observed well.

CEREBROSPINAL GANGLION. The structure of the ciliary ganglion is absolutely different from the cerebrospinal sensory ganglia in which uni- and mainly bi-polar cell types are dominant (Plate III, 3). The T-shaped bifurcation of the cells that is particularly characteristic in the higher vertebrates here is very rare. The two processes originate generally from the two opposite poles. The size of cells is large, as a rule, between 35—50 μ, being almost double of the size of the ciliary ganglion cells. A characteristic property of the cells is the great differ-

ence appearing in their staining. Apart from dark impregnated cells, there are frequent also neurons stained light yellow. The number of dark-stained cells is always higher. These cells as well their processes and the thick myelinated fibres running in the ganglion are verifying doubtless tehir belonging to the cerebrospinal system.

SYMPATHETIC GANGLIA. The ciliary ganglion of fishes are not similar structurally to the ganglia of the ganglia of the cranial sympathetic system, either. Having investigated the five pairs of cranial ganglia of the carp, we established that the most important feature of their histologic structure is the difference in the sizes of the cells. Beside the large cells of the diameter of 25—30 μ, groups of thoroughly small cells of 8—10 μ take place (Plate III,4). The body of the larger and smaller nerve cells is similarly round, with a granular plasma pattern. The cell process appears but rarely. Thus, a great part of cells can probably be classified into the unipolar type. After prolongated impregnation the large cells have two, resp. even three processes. The cells of smaller or larger size appear alike in an identical colour (Plate II, 2). There aren't among them any cells stained very dark. Also in the nerve trunks between the ganglia, there are some nerve-cells classified into the small cells as to their size. The nerve fibres have Schwann-sheaths. The Schwann's *nuclei* in the cranial ganglia of the carp are particularly long, 15 μ. In the nerve-trunks we do not see any fibres stained stronger, resp. of thick sensory character. Round the cells glial cell *nuclei* are located. Around the larger cells, these *nuclei* are larger and longish. As in the vegetative ganglia generally, a great number of synapses appear also here, i.e., preganglionic fibre-ends. These terminations appear generally on the large cells or in the immediate vicinity of them. The termination in the form of a dark stained end-head or end-bulb is always stained black, in that way being well separated from the glial cell *nuclei*. These synapses can be found in great number in the cranial sympathetic ganglia.

It is referred to by the comparative structural differences that the structure of the ciliary ganglion of fishes is peculiar. It does not show any similarity to the cerebrospinal or the sympathetic ganglia. The cause of the differences can be looked for as evolutionary deviations, in the origin from the mesencephalon and, as a result of that, in their exclusively close connection with the oculomotoric nerve.

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PLATE II

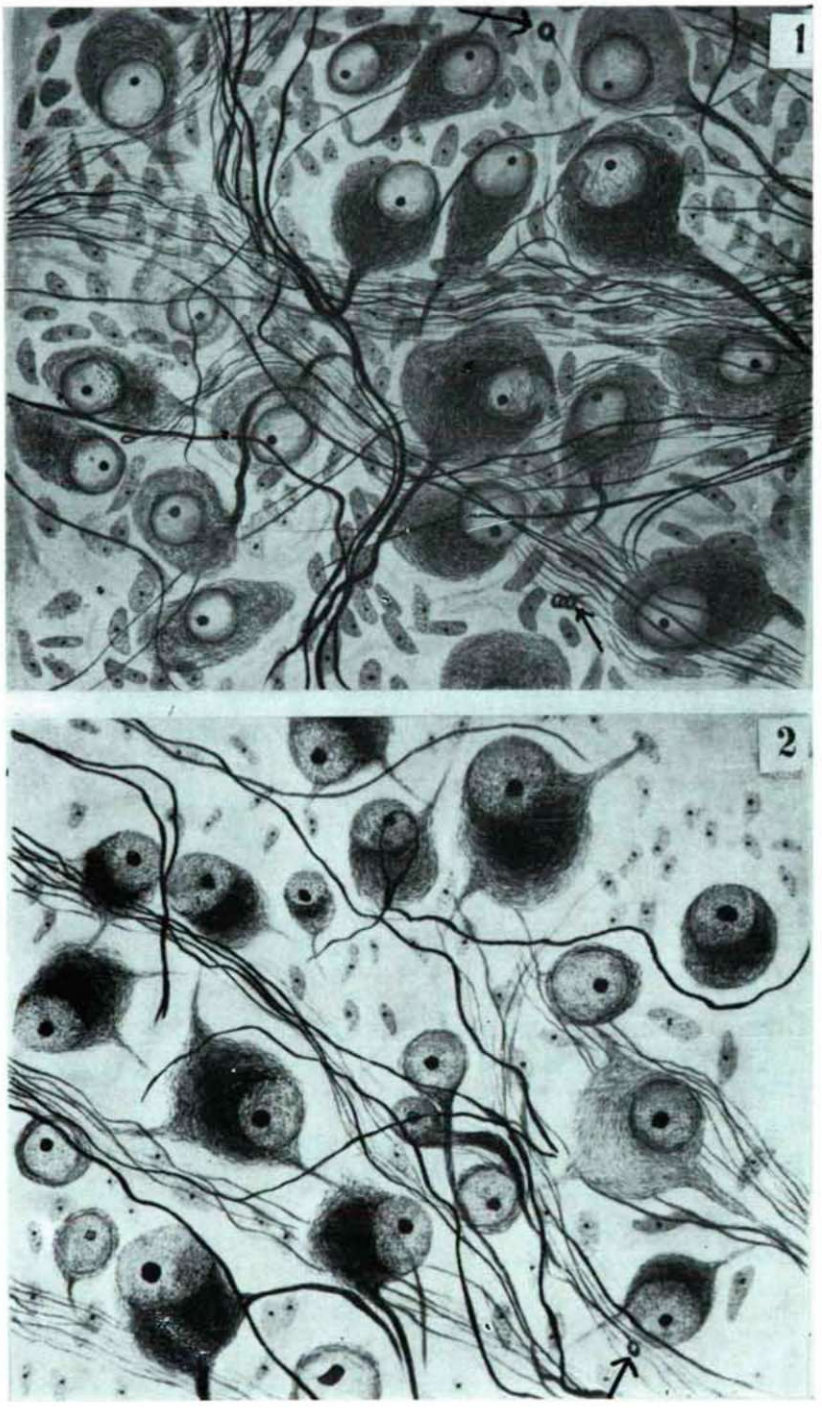

PLATE III

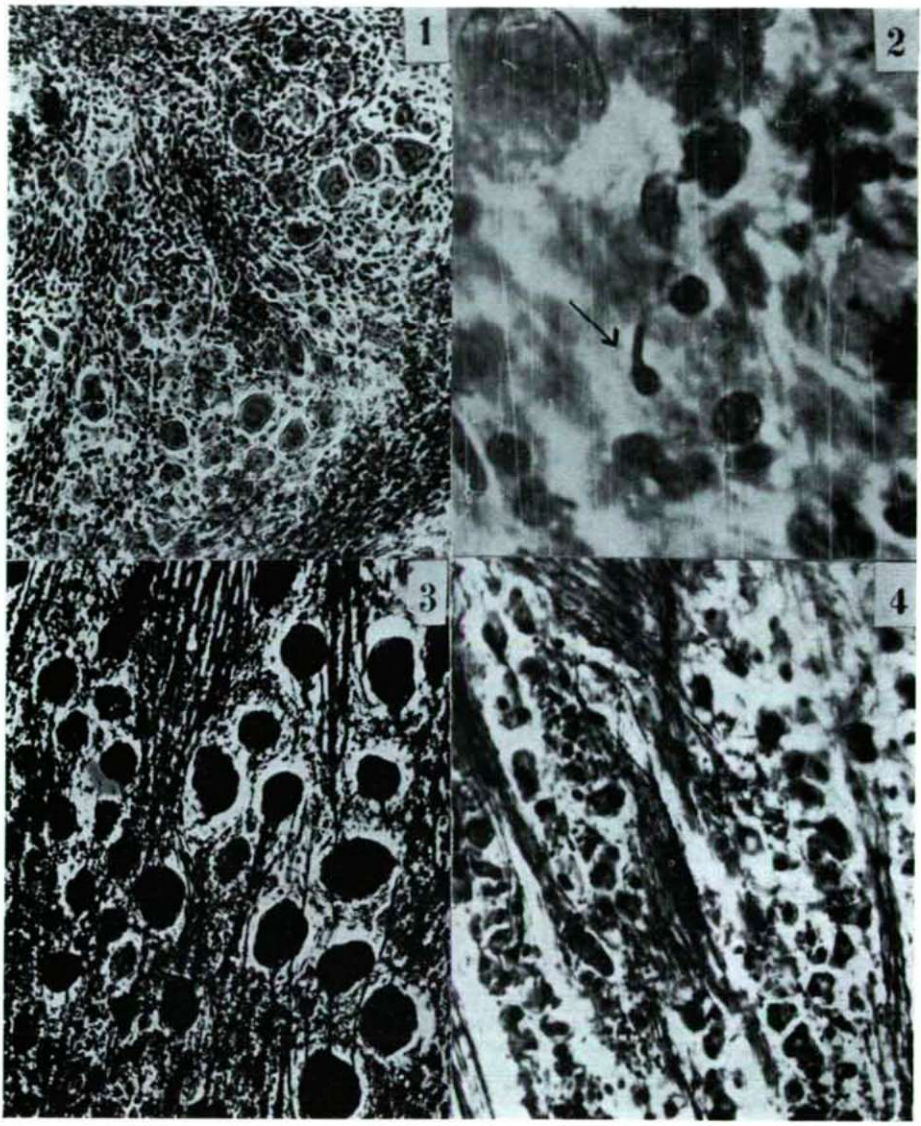

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Plate II
Fig. 1. *Cyprinus carpio.* The structure of the ciliary ganglion. end-ring.Magn. 600 x.
Fig. 2. *Cyprinus carpio:* The structure of the II. Cranial Sympathetic ganglion. end-ring. Magn. 600 x.

Plate III
Fig. 1. *Cyprinus:* The structure of ciliary ganglion.
Fig. 2. *Cyprinus:* End-bulb among the ciliary cells.
Fig. 3. *Cyprinus:* The structure of ganglion opthalmicum profundum.
Fig. 4. *Cyprinus:* The structure of II. Cranial sympathetic ganglion.

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CAMPTOCHIRONOMUS HUNGARICUS, A NEW CHIRONOMUS SPECIES

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Introduction

In Hungary, since 1945, rice has been grown in a larger and larger area. Growing is taking place first of all and almost exclusively in alkali soils for the following reasons:

They preserve well the inundation water; other culture plants do not grow there because of the high Na_2CO_3 content, they can, therefore, be employed profitably exclusively in that way, apart from the possibility of increasing and extending the cultivation without decreasing the areas of growing other culture plants. Thus the sodic soils can be made use of, owing to growing rice.

The high salt content of the alkali areas used in growing make possible only the cultivation of rice in the given area for several years, assuring anyway favourable conditions to parasites breeding rapidly. The rice-field fauna has developed from the animal kingdom of stagnant waters of the adjacent territories as the rice-fields were inundated from these. The euryhalinic and eurythermous animal species of the standing waters grow to be dominant species in the rice-fields of sodic soil. The high degree accomodation is manifested also in the alimentary accomodation in the course of which several animal species, indifferent before, have become the parasites of rice (Megyeri, 1960).

It is clear from the literature in this country that the parasites are in the way to be cleared up and it is under elaboration, too, how to be protected against them (Szekér, 1953; Berczik, 1957; Bognár, 1957; Megyeri, 1960; Szilvássy, 1960).

In 1963, a systematic faunistical investigation of the rice-fields and backwater in the environs of Szarvas began and, as a result of that, a new *Chironomus* species was found in 1967.

Description of habitats

At both banks of the backwater, *Phragmitetum* associatic is dominant. In the irrigation canal there isn't any fully developed association but some monocotyledonous weeds are living there, as well. Larvae can be found embedded in mud.

The backwater is lying at Szarvas, in an alkali area, and the irrigation canal — where the investigations were carried out in the neighbourhood of Békésszentandrás (west of Szarvas) — is in an alkali area, as well. Its water has come from the backwater.

Methods

In the areas mentioned above we have performed observations and, as far as possible, collections, too, fortnightly in every season. From the mud, there were picked samples with a 10×10×5 cm grabber. From early spring till late autumn, we have netted from the swarming imago, netting also the plants covering the rice-fields and their environment, as well the riparian plants of the backwater.

Results of investigations

In the course of our collection, on the first of May 1967, we could find one pupa, on the 21st of May three imagos in the mud of the backwater, and on the 29th of November five larvae in the irrigation canal. The larvae were grown to be imago in laboratory culture vessels.

Characterization of the species:

Larvae: 11—15 mm long, light red, living in the mud of stagnant or slow- flowing water. Its food is: root of monocotyledonous plants and necrotic plant tissues. It consumes first of all the living particles. During winter, they survive in the wet mud even without being covered by water. From most of the larvae frozen in the mud, there develop imagos in spring. They cannot be separated from the other larvae of *Camptochironomus* sp. On February 6th and February 16th 1968, we collected larvae from the mud frozen through.

Pupa: It is 11—13 mm long. It cannot be separated from pupas of related species on the basis of its marks.

Imago: 9—12 mm. The male is yellowish-red, the mesonotum with brownish-yellow stripes. The posterior part of metanotum is dark brown. Its characteristics are a grey haltera, yellowish pulvillum developed moderately. Its tarsus is short, covered with dense bristles.

Its abdomen is yellowish-green, the tergit of segments nos. 6—8 is brown. The sternits of segments nos. 2—5 are gradually elongated, so the abdomen widens and thickens more and more, then at segments nos. 6—8 it grows quickly narrower. Thus the body of a male of the animal species is similar to the fuselage of a helicopter. AR=4. Its wings are opaque glass-like, r-m is black. LR=1,2.

The appendix of the hypopygium dorsalis is much longer than the apex of appendices derived from the bilateral excises. The appendix of the dorsal lamella first becomes narrower near to its end and then it again grows wider.

Appendix no. 1 of the hypopygium is rudimentary but perceptible, its second appendix is, however, well-developed.

Swarming: in April, May, October, in a mild autumn also in November.

We have named the species described *CAMPTOCHIRONOMUS HUNGARICUS.*

On the basis of nutritive biologic investigations performed on larvae of the species, we have established that they consume both living and dead parts of plants. It is proved by feeding experiments carried out with germinated rice that they gnaw the roots of young rice. The larvae can be grown imagos exclusively on that food. There has increased, therefore, with this species not only the number of known species of animals

Fig. 1. *Camptochironomus Hungaricus* nov. spec. Hypopygium (original).

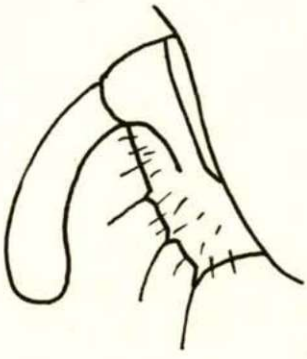

Fig. 2. The 1. extension (original).

but also that of the rice parasites recognized. So long we have not learned the presence of larvae in large numbers. With full knowledge of their nutritive biology, however, their pullulation in the rice-fields is to be expected, thus one has to reckon with the damage of the larvae of this species, as well, in the future. It is more and more urgent, therefore to elaborate an adequate way of defence against the larvae of this *Chironomus* species.

Summary

In 1967, in the course of the systematic faunistical investigations of the rice-fields adjacent to Szarvas and the backwaters of the Triple Kőrös at Szarvas, in connection with the species *Chironomus,* we observed a new *Chironomus* species in the shapes of larvae, pupae, and imagos. On the basis of nutritive biological laboratory investigations on larvae, it came to light that they feed on living and dead monocotyledonous plant particlese. It has been proved, as well, that they consume

also the roots of germinating rice, and even they can grow igamos exclusively on that food. The larvae survive in winter, frozen in a wet mud, even without being covered by water, as it was proved by the collections performed in February.

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Fig. 3. The damage of *Camptochironomus Hungaricus* grub on the rice roots, which are totally ruined (original photo).

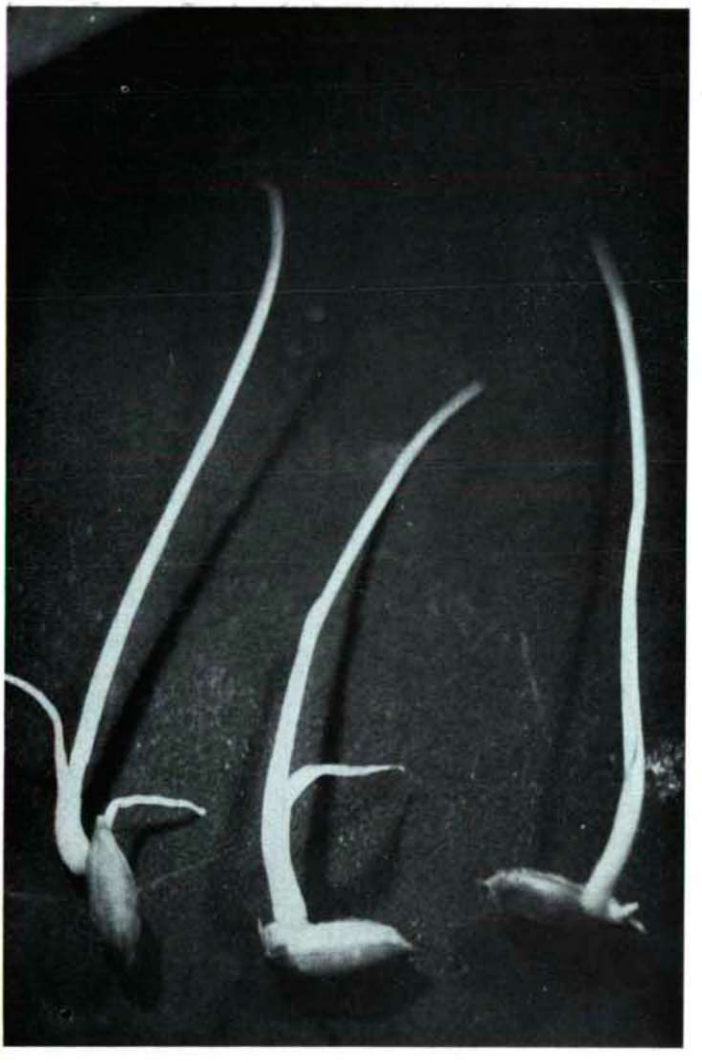

Fig. 3

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